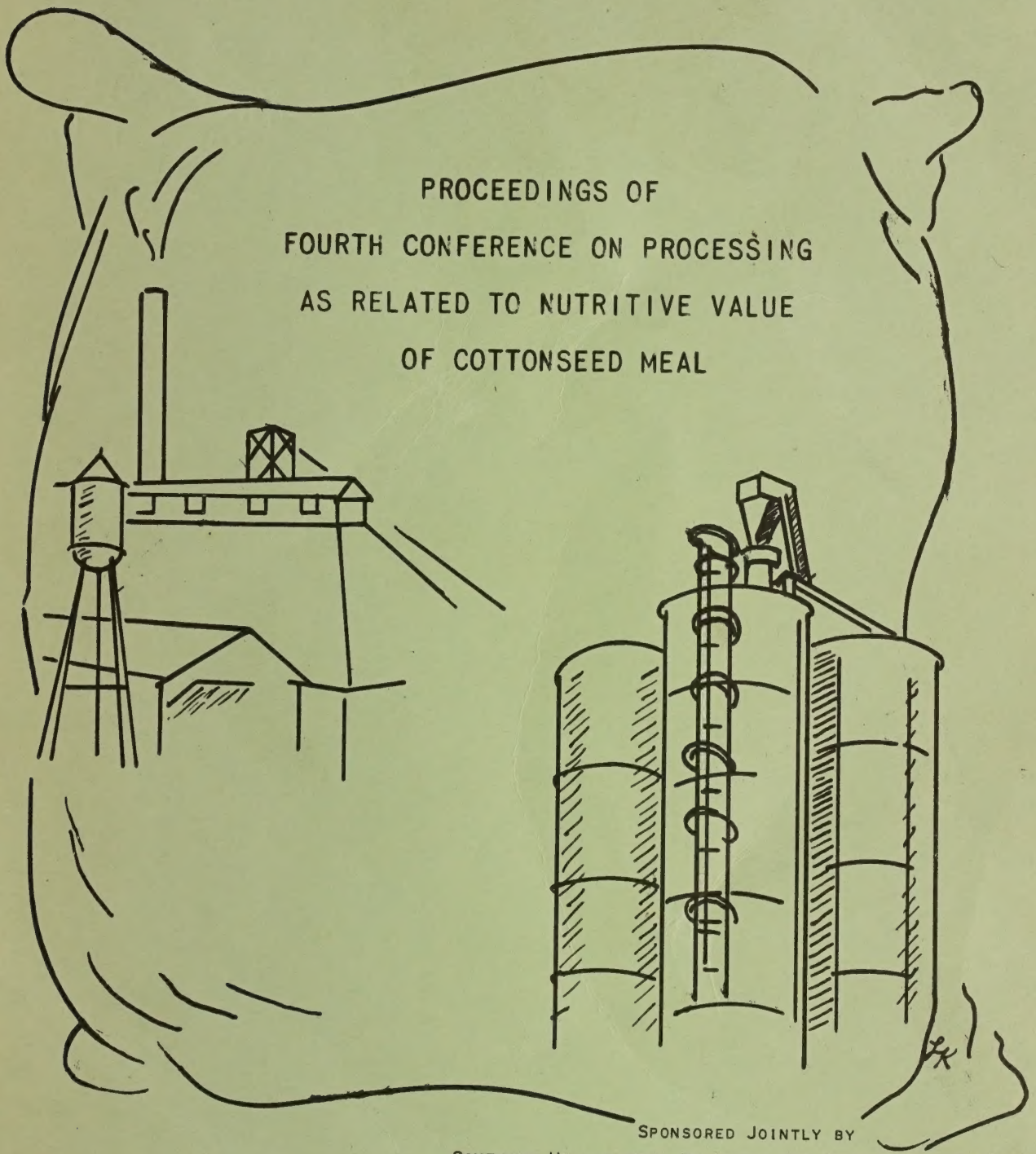
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PROCEEDINGS OF FOURTH CONFERENCE ON PROCESSING AS RELATED TO NUTRITIVE VALUE OF COTTONSEED MEAL

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SOUTHERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION
AND
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NATIONAL COTTONSEED PRODUCTS ASSOCIATION

JANUARY 14-16, 1957
NEW ORLEANS, LOUISIANA

FOREWORD

This report contains highlights of the information presented at the Fourth Conference on Processing as Related to the Nutritive Value of Cottonseed Meal, sponsored jointly by ARS Southern Utilization Research and Development Division and the Educational Service and Research Committee of the National Cottonseed Products Association. The conference was held at the Southern Regional Research Laboratory, Southern Utilization Research and Development Division, in New Orleans, Louisiana, January 14-16, 1957.

The participants at this conference met to review the information obtained in feeding studies designed to determine the practicality of using various cottonseed meals, representative of all types of commercially processed meals, in rations for poultry, swine, and ruminants. Such information is necessary for determining the most desirable meal properties and processing conditions for obtaining optimum growth and feed efficiency.

Abstracts of papers presented at this conference, reports and recommendations of appointed Committees, and a summary of the entire conference are included in this report. Further details may be obtained by writing to the Southern Utilization Research and Development Division, P. O. Box 7307, New Orleans 19, Louisiana, or to persons listed on the program.

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CONTENTS

Page No.

Interpretative Summary of Conference
A. M. Altschul 1
Summary of Committee Reports and Recommendations
L. S. Bensabat 9

OPENING REMARKS

Welcoming Address
C. H. Fisher 14
Response
R. Stokes 15
The Cottonseed Crushing Industry's Interest in Research
A. L. Ward 16
Trends in the Use of Cottonseed Meal for Livestock in The South
C. W. Upp 18
Current Cottonseed Research Program
H. L. Wilcke 19
Summary of History of Cottonseed Meal Collaborative Conferences
A. M. Altschul 21

Seminar I - REPORT ON COLLABORATIVE STUDY OF COMMERCIALLY PROCESSED COTTONSEED MEALS

Introduction to Collaborative Study
A. M. Altschul 22
Presentation of Analytical Data on Cottonseed Meals
B. Piccolo 23
Interpretation of Results
V. L. Frampton 31
Amino Acid Supplementation to Broiler Feeds
O. C. Bucek 34
Study of Commercial Cottonseed Meals in Chick Rations
W. L. Hunter, V. P. Entwistle and W. C. Green 36
The Effect of Processing on Nutritive Value of Cottonseed Meal
E. L. Stephenson 39
Nutritive Value of Commercial Cottonseed Meals
P. D. Aines 39
Commercial Cottonseed Meals in Chick Broiler Rations
A. Heidebrecht 41
Effect of Processing and Dietary Level of Cottonseed Meals on Chick Performance
R. J. Lillie and C. A. Denton 42

Page No.

Cottonseed Meal Nutritive Quality
H. R. Halloran, B. Maxwell and J. B. Lyle 43
Nutritive Quality of Cottonseed Meal Rations for Chicks
J. E. Corbin and H. L. Wilcke 46
Cottonseed Meal-Hull Bran Studies
A. B. Watts and C. M. Sasser 46

Seminar II - PROTEIN QUALITY AND GOSSYPOL TOXICITY

Toxicity of Free Gossypol in Relation to Protein of Feed
F. H. Smith 57
Effect of Protein Source and Gossypol Level in the Ration on Growing Pigs
A. J. Clawson and E. R. Barrick 61
Gossypol Toxicity and Protein Level in the Nutrition of the Chick and Laying Hen
R. Narain, C. M. Lyman and J. R. Couch 65
Toxicity and Protein Quality Studies on Cottonseed
E. Eagle 66
Relation of Quantity of Dietary Protein to Tolerance of the Growing Pig for Free Gossypol
I. P. Earle and J. W. Stephenson 66
Effect of Varying Protein Intake, Source and Lysine Content on Free Gossypol Tolerance by the Rat
C. A. Cabell 67
Protein Quality Evaluations of Coded Cottonseed Meals in the Rat and Chick
E. Eagle 71

Seminar III - CHEMICAL MEASURES OF NUTRITIVE VALUE

Lysine Content of Cottonseed Meals
W. H. Martinez and V. L. Frampton 74
ε-DNP Lysine Determinations in Cottonseed Meals
E. J. Conkerton and V. L. Frampton 76
Reaction of Gossypol with Chemicals
W. H. King 79

Seminar IV - EFFECTS OF COTTONSEED PRODUCTS ON EGG QUALITY

Status of Research on Egg Yolk Discoloration in Relation to Gossypol
C. R. Grau 81

Page No.

Incorporation of Gossypol into the Eggs of Hens Fed Gossypol Schiff Bases
J. M. Dechary 83
Effect of Cottonseed Oil on the Discoloration of Stored Shelled Eggs
R. J. Evans 85
Factors in Cottonseed Meal that Cause Pink Discoloration in Cold Storage Eggs
A. J. Kemmerer and J. Masson 88

Seminar V - HISTOPATHOLOGICAL EFFECTS OF GOSSYPOL

Histopathological Effects of Gossypol
T. M. Ferguson, R. Narain, E. M. Banta and J. R. Couch 93
Histopathological Effects of Gossypol Fed to Pigs
E. L. Stephenson 94

Seminar VI - RUMINANT STUDIES WITH COTTONSEED MEAL

The Effect of Protein Intake on Reproductive Performance, Gains and Blood Constituents in Beef Cattle
A. G. Warnick, E. Bedrak, M. Koger, J. F. Hentges,Jr. and T. J. Cunha 96
Utilization of Protein and Non-Protein Nitrogen by Lambs
W. R. Woods and A. D. Tillman 98

COMMITTEE REPORTS

Report and Recommendations of Committee on Poultry 103
Report and Recommendations of Committee on Swine and Ruminants 105
Report and Recommendations of Committee on Chemical Measurements and Nutritive Value 106
Report and Recommendations of Committee on Effect of this Research on Industry Development 108

APPENDIX

Attendance List 109

Page No.

Incorporation of Gossypol into the Eggs of Hens Fed Gossypol Schiff Bases
J. M. Deatherage 83
Effect of Cottonseed Oil on the Discoloration of Stored Shelled Eggs
R. J. Evans 85
Factors in Cottonseed Meal that Cause Pink Discoloration in Cold Storage Eggs
A. J. Kemmerer and J. Mason 88

Seminar V - HISTOPATHOLOGICAL EFFECTS OF GOSSYPOL

Histopathological Effects of Gossypol
T. M. Ferguson, P. Harwin, K. M. Pantz and J. R. Couch 93
Histopathological Effects of Gossypol Fed to Pigs
E. L. Stephenson 94

Seminar VI - RUMINANT STUDIES WITH COTTONSEED MEAL

The Effect of Protein Intake on Reproductive Performance, Gains and Blood Constituents in Beef Cattle
A. D. Warnick, E. Bedrak, M. Koger, J. F. Hentges, Jr. and T. J. Cunha 96
Utilization of Protein and Non-Protein Nitrogen by Lambs
W. R. Woods and A. D. Tillman 98

COMMITTEE REPORTS

Report and Recommendations of Committee on Poultry 103
Report and Recommendations of Committee on Swine and Ruminants 105
Report and Recommendations of Committee on Chemical Measurements and Nutritive Value 106
Report and Recommendations of Committee on Effect of this Research on Industry Development 108

APPENDIX

Attendance List 109

INTERPRETATIVE SUMMARY OF CONFERENCE

A. M. Altschul
Southern Regional Research Laboratory
New Orleans, Louisiana

INTRODUCTION

This is the fourth of a series of public conferences held at this Laboratory to review research on cottonseed processing as related to the nutritive value of the meal; the first three conferences were held in November, 1950; November, 1951; and November, 1953. These conferences arose because there had been existing since about 1950 a broad cooperative program on the effect of processing on the nutritive value of cottonseed meal for non-ruminants involving laboratories of the U. S. Department of Agriculture, of State Experiment Stations, of commercial institutions, and involving extensive cooperation with the Research Committee and the Educational Service of the National Cottonseed Products Association. Some of the work reported at these various meetings was supported in part by fellowships given by the National Cottonseed Products Association.

Although this program, comprehensive in size and scope, was never coordinated officially, the effect of these conferences has been to achieve a high degree of coordination through exchange of information and to offer a mechanism for arriving at a consensus on each of the problems involved. Because of the almost complete representation of all interested parties, these meetings have assumed a somewhat semi-official character and have exercised a profound influence on the thinking of the industry, the producers of the meals and their users, as well as on those conducting research.

The meeting was opened by A. M. Altschul who was chairman of the local committee. The group attending the conference was welcomed by C. H. Fisher, Director of the Southern Utilization Research and Development Division. He was followed by Robert Stokes who acted as representative of the National Cottonseed Products Association. The interest of the Educational Service was emphasized by remarks written by A. L. Ward and read by Garlon Harper. The interest of the Research Committee of the National Cottonseed Products Association was affirmed by its chairman, H. L. Wilcke. He also appointed various committees to report at the last session on the status of research and development in the various fields.

At one of the luncheons of the conference, the featured speaker was C. W. Upp, Director of Louisiana Agricultural Experiment Station and formerly Head of the Poultry Industry Department at Louisiana State University.

SEMINAR I

Collaborative Study on Cottonseed Meals

Considerable progress had been made in understanding the processing variables which affect the nutritive quality of cottonseed meals. Meals suitable for inclusion in diets of non-ruminants have been produced; indeed, in 1954 approximately 250,000 tons of cottonseed meals were used in poultry and swine feeds, and in later years the figure may have increased to 500,000 tons. Having established this market for cottonseed meals, it was deemed necessary to examine present commercial processing methods critically by comparing the relative merits of meals produced under typical commercial conditions. The Research Committee of the National Cottonseed Products Association, accordingly, suggested that it would be helpful to evaluate present commercial cottonseed meals for their use in poultry and swine diets. A fellowship was supported at the Southern Regional Research Laboratory to help carry out this cooperative effort, and a plan was prepared by the Cottonseed Meal Subcommittee of the Research Committee and circulated to interested groups in state and federal institutions and in industry. The Southern Regional Research Laboratory was given the responsibility of making all arrangements for this study. The purpose of this study was stated as follows:

> "To provide information regarding the suitability and performance of mixtures of commercial cottonseed meal with soybean meal in practical poultry and swine rations and to provide additional information on the relationship between processing conditions and protein quality."

Accordingly, cottonseed meals produced by the various processing methods were selected on the basis of their free gossypol content and protein solubility in 0.02 Normal aqueous sodium hydroxide solution. Among the meals selected for study were: three screw-press meals representing light, medium, and extreme processing conditions; three prepress solvent-extracted meals representing various levels of intensity of processing; one direct solvent-extracted meal; one hydraulic-press meal; and one solvent-extracted, chemically-treated meal.

The reference diet used was that recommended for broilers by the National Research Council. Experimental diets were prepared by replacing 50, 75 or 100% of the soybean meal of the ration with cottonseed meal, on a nitrogen basis. The Research Council of the American Soybean Association provided a standard soybean meal (52% protein) to be used as control ration in the experiments and to be mixed in various proportions with the cottonseed meals. The performance and growth of test animals given rations containing cottonseed meals were to be compared with those of animals fed control ration containing soybean meal alone as the protein supplement. All samples were collected and the diets mixed in one operation through the help of the Ralston Purina Company. Diets and meals were submitted to all of the investigators who volunteered to study the nutritional qualities of these meals.

After an introduction describing the purposes and the general arrangement of the collaborative study by A. M. Altschul, there followed a presentation of analytical data on the cottonseed meals and rations by B. Piccolo and a tentative interpretation of the results based on some preliminary analyses of the data by V. L. Frampton.

These initial discussions of the chemical analyses of the meals were followed by specific discussions of the results of nutritional investigations on the diets containing these meals. The general results of this experiment might be summarized as follows: There is a variation in the chemical composition of cottonseed meals even within a given process of manufacturing. Chemical properties of a cottonseed meal cannot be predicted on the basis of the process used, but are a function of processing conditions. The free gossypol content of meals tested and prepared by the screw-press process, prepress solvent-extracted process and by solvent-extraction followed by chemical treatment was 0.04% or lower. Meals in this study with a free gossypol content higher than 0.04% were produced by the hydraulic-press and direct solvent-extraction processes. Aside from one cottonseed meal in the group, all the meals analyzed had a nitrogen solubility of less than 75%. The lysine content of the meal indicated a variability from a high of 4.2 grams of lysine per 16 grams of meal nitrogen for an unheated meal prepared in the laboratory from prime seed to a low of 2.3 grams for a heat-damaged commercial meal. These two meals were not used in this nutritional study. Most of the values for lysine content of commercially prepared cottonseed meals used in this study ranged between 3 and 4 grams per 16 grams of nitrogen.

It was recognized that for proper interpretation of the data it would be necessary to make a complete statistical determination. However, from a limited analysis, certain tentative conclusions were reached. The lysine content of the commercially prepared cottonseed meals was of prime importance in determining the growth response and the feed utilization by growing chicks. A marked improvement was observed

in the growth of broilers when the rations containing cottonseed meal were supplemented with lysine. The total gossypol content of the meal was also of major importance and at high levels the presence of gossypol in the free or combined state had a marked detrimental effect. The crude fiber in the meals had a detrimental effect, but this was smaller by comparison with the effect of total gossypol. None of the commercially-prepared cottonseed meals used in this experiment produced as rapid a growth response in chicks or yielded as efficient feed utilization as did the soybean meal used alone as the reference protein supplement. The rations containing cottonseed meals were higher in fiber content than the control soybean ration. Cottonseed meal rations also decreased in nutritive quality upon storage.

Improvements in growth response and feed utilization were obtained when cottonseed meal was mixed with soybean meal. Some of the mixtures containing equal amounts of cottonseed meals and soybean meals were equal in performance to soybean meal.

These conclusions were summarised in the reports of several Committees.

SEMINAR II

Protein Quality and Gossypol Toxicity

Previous efforts to define in rations the level of free gossypol which would not be toxic to swine have met with little success. There were variations in defined levels from one experiment to another. This indicated the presence of at least one other variable not dependent on free gossypol which influenced the results. Similar difficulties have been encountered in determining the level of gossypol and cottonseed toxicity to rats, and these are underscored in the presentation by E. Eagle.

Evidence is now accumulating that the level and quality of the protein in the diet has an influence on toxicity of gossypol. With higher quality meals, or with higher levels of protein in the diet, it is possible to reduce the toxicity of gossypol. Evidence for this fact was presented both in rat studies, in which other sources of protein such as casein and soybean protein were substituted for cottonseed meal, as well as in studies employing various levels of cottonseed meal.

In one experiment presented by A. J. Clawson and E. R. Barrick, a level of free gossypol of 0.018% in the diet did not effect growth and showed no evidence of toxicity when soybean meal was the source of protein.

SEMINAR III

Chemical Measures of Nutritive Value

A discussion of the lysine content of the cottonseed meals analyzed by the Moore and Stein chromatographic procedure was given by Mrs. W. H. Martinez; the determinations for total lysine were made after acid hydrolysis of the meals. It was reported that the lysine content of the cottonseed meals prepared by various processing procedures varied, and those procedures which involved the greatest amount of heat damage produced meals having the lowest lysine concentrations.

It is now possible to analyze for unreacted lysine by determining the free epsilon amino group of lysine in the protein. Such lysine would presumably be lysine in which the epsilon amino group has not reacted during processing, and presumably is more available for nutritive use by the animal. This epsilon amino group of lysine can be determined on pure proteins by reaction with dinitrofluorobenzene. There have been some reports that the epsilon amino group of lysine can also be determined in animal proteins by such a procedure, but no reports have been given of its application to vegetable proteins. At this meeting there were the first reports by E. J. Conkerton on the application of this method to cottonseed meal and a parallel report by B. P. Baliga. This method requires less effort than the method for determination of total lysine and may more closely approximate the "available" lysine in the meal.

SEMINAR IV

Effects of Feeding Cottonseed Products on Egg Quality

A review of the relationship between gossypol and egg yolk discoloration was presented by C. R. Grau, who stated that gossypol fed to laying hens is deposited in the yolk as gossypol-cephalin or as gossypol-protein. He uses the amount of gossypol-cephalin in the egg as a measure of dietary gossypol that is available to the hen, and as a basis for determining which types of diets will cause discoloration of the egg yolk. When gossypol is heated with strong alkali it loses its carbonyl groups and becomes apogossypol which does not form Schiff bases with amines such as ethanol-amine, but may still be incorporated into eggs and probably combines with protein. The phenolic groups of apogossypol and its oxidation products appear to be responsible for the yolk discoloration; when these groups are blocked by forming methyl ethers discoloration does not occur when the eggs are exposed to an ammonia atmosphere.

A most promising approach to solving this problem is to prevent the absorption of gossypol from the intestinal tract. Certain long chain aliphatic amines prepared by J. M. Dechary and fed to laying hens by C. R. Grau apparently were not absorbed by the hen and did not produce egg yolk discoloration.

W. H. King described a method in Seminar III for determining the gossypol content of meals and of differentiating it from the gossypol-like materials that are also analyzed for in the conventional analytical procedures for determining gossypol. Since only small smounts of free gossypol are needed to produce egg yolk discoloration, a discrepancy between physiologically active gossypol and materials that show up as gossypol might be important.

Beside the egg yolk discoloration, there is another lowering of egg quality that is due to cottonseed products; this is the so-called pink white. R. J. Evans described attempts to fractionate crude cottonseed oil to isolate the material which causes the pink whites. Similar work was reported by A. R. Kemmerer and J. Masson. They reported that when crude cottonseed oil was fed as 3 or 6% of the diet, pink discoloration resulted. Further fractionation tests seemed to indicate that these factors are in the fatty acid fractions of the crude cottonseed oil. An attempt was made to correlate the Halphen test with the presence of pink discoloration factors in cottonseed oils. The results as yet are not clear cut in showing any relationship between the pink albumins produced and the material in cottonseed oil which gives the so-called Halphen test. Research is underway toward either synthesizing or isolating this material from cottonseed oil in order to determine its role in the egg discoloration problem.

With the accumulation of information on the nature of the materials that cause egg yolk and egg white discoloration, the way is open for experiments to eliminate these difficulties in a practical manner. The egg yolk problem is by far the most important, since the discoloration is the limiting factor in the more extensive use of cottonseed meal in poultry rations. Chemical treatment does seem to offer the possibility for solving this problem. The pink albumin problem is less important economically, since it seems to arise from the presence of oil in the meal. Since most meals have a low oil content, this problem is less serious. It appears, however, as a problem when cottonseed foots and soapstock are added to meals in an effort to increase their oil content, energy value, and improve their physical properties.

SEMINAR V

Histopathological Effects of Gossypol

Preliminary observations on the effect of feeding gossypol, in the form of pigment glands from cottonseed and as pure gossypol, to hens and

baby pigs indicate the possibility of finding considerable information about the mode of action of this material.

Ferguson, et al, found that when isolated cottonseed pigment glands were fed to laying hens there was a breakdown of the ova in the ovary with the rupture of egg yolks, frequently accompanied by hemorrhage of the follicle.

Stephenson found abnormalities in the kidneys of pigs fed isolated gossypol. Feeding of isolated pigment glands produced pathologic lesions in several organs.

SEMINAR VI

Ruminant Studies with Cottonseed Meal

Some studies were reported on the effect of feeding cottonseed meal to ruminants and on the difference, if any, between nutritive quality of cottonseed meal as measured by non-ruminant feeding tests and its use for ruminants. There seems to be little difference between cottonseed meals of high or low nitrogen solubility in their ability to produce gains in lambs. In these particular experiments digestibility of cottonseed meal protein was lower than that of soybean or sesame meal protein. This work is continuing.

Resolutions and Reports

The meeting concluded with reports of various committees appointed to try to develop a consensus on the various subjects discussed. The highlights of some of the recommendations are as follows:

1. It was recommended that nutritional data obtained in the large-scale cooperative experiment be subjected to complete statistical analysis before any conclusions be drawn about the relationship between processing conditions, chemical properties, and the nutritive performance of cottonseed meals in broiler rations.

2. The conference reaffirmed the validity of the statement on cottonseed meal made at the previous conference (Nov., 1953) but suggested that there is now evidence showing certain cottonseed meals of 0.04% free gossypol with less than 75% nitrogen solubility, which, when substituted for 50% of soybean meal in a practical broiler ration, give excellent performance. The committee felt, however, that it should be the objective of cottonseed processors to retain the maximum quantity of desirable nutrients insofar as possible.

3. Research was urged on testing of 50% protein cottonseed meals in practical rations and for continuing the work on lysine analysis of cottonseed meals, on the reactions of cottonseed proteins with gossypol and other materials present in the meal, and on the work on egg yolk and pink white discoloration problems.

4. The available data indicated that a dietary level of 0.0045% free gossypol can be fed to swine without evidence of toxicity and that a dietary level of 0.0066% is the lowest reported to be toxic. The tolerance of growing swine for gossypol is influenced by protein quality and the level of dietary protein. Work was urged to determine the gossypol tolerance of animals under well-defined conditions.

5. Evidence was presented that cottonseed meal can be fed with satisfactory results as the only source of supplementary protein for growth and reproduction of ruminants.

6. There is evidence that there are a number of chemical determinations which tend to measure differences in nutritive value of cottonseed meals, but none of these gives an entirely satisfactory evaluation when used as the sole criterion. In those cottonseed meals in which free gossypol is low, the bound gossypol is considered an important factor. In those cottonseed meals which have a high free gossypol content, the free gossypol was considered an important factor. For intermediate levels of nitrogen solubility there is no direct relationship between this criterion and nutritive value. It was recommended that work be continued, particularly on lysine analyses, and the relationship of the data obtained to processing conditions and nutritive quality be established.

SUMMARY OF COMMITTEE REPORTS AND RECOMMENDATIONS

L. S. Bensabat
Southern Regional Research Laboratory
New Orleans, Louisiana

I. Cooperative Nutritional Experiment on Cottonseed Meals

A. Purpose of Experiment

"To provide information regarding the suitability and performance of mixtures of commercial cottonseed meal with soybean meal in practical poultry and swine rations, and to provide additional information on the relationship between processing conditions and protein quality."

B. Procedure

After screening tests on cottonseed meals supplied by processors throughout the country, large quantities of cottonseed meals which were considered to be most representative of commercial production were purchased by the NCPA. The screening involved tests for total and soluble nitrogen as well as tests for free and total gossypol. The selected meals were shipped to St. Louis where their incorporation into a basal broiler ration was supervised by L. D. Keller of the Ralston Purina Company and by Dr. Frampton and Mr. Piccolo of the SURDD. Cottonseed meal as a protein supplement was prepared for use alone and in combinations of 50:50 and 75:25 ratios of cottonseed to soybean meal containing 52% protein. A ration in which the soybean meal alone supplied the protein supplement of the diet was also prepared for use as a control. Efforts were made to prepare the rations in such a way that the protein level was the same in all the diets. At the Southern Regional Research Laboratory samples of each meal and each ration were then subjected to exhaustive chemical analyses, involving tests for free and total gossypol, inorganic and organic phosphorous, total fat, total and reducing sugars, lysine, crude fiber, and total and soluble nitrogen.

Six tons of the cottonseed meals were shipped to two stations where hog feeding studies were carried out, and sixty tons of the poultry rations were supplied to stations in Arkansas, Arizona, California, Florida, Georgia, Louisiana, Michigan, Mississippi, and Texas for nutritional evaluation on a total of 12,000 growing chicks. This comprised one of the largest nutritional experiments ever conducted on broilers. In addition, cottonseed meals were supplied to experimenters in Florida and Oklahoma for evaluation as a sole source of protein for cattle and lambs. Samples of the cottonseed meals were also shipped to the Rowett Institute in Scotland for protein evaluation studies.

C. Conclusions

When the statistical analyses of the results are completed, information should be available on the suitability of present commercially produced cottonseed meals as protein supplements for broiler rations. It is possible that data will be available on the effect of total and free gossypol, fiber, nitrogen solubility, lysine, and other constituents on the nutritive performances of the meals alone, and in mixtures with soybean meals, in practical broiler rations. There should also be provided information on the comparative performance of these meals in various stations and after various periods of storage. Precluding the results obtained from such an analysis, the following general conclusions were reached by the different committees:

1. Chemical Composition

There is a variation in the chemical composition of meals within a given process.

a. The free gossypol content of meals tested prepared by screw-press process, pre-press solvent extraction process, and solvent extraction followed by chemical treatment is 0.04% or lower.

b. Meals used in the nutritional experiment with free gossypol content higher than 0.04% were produced by the hydraulic press and straight solvent extraction processes.

c. Aside from one meal in the group, all of the meals analyzed had a nitrogen solubility less than 75%.

d. Chemical properties of the meal cannot be predicted on the basis of the process used, but are a function of the processing conditions.

II. Chemical Measurements of Nutritive Value

A. Gossypol

Increasing levels of total gossypol (0.62% to 1.11%) have a detrimental effect on the rate of growth of chicks.

1. Bound gossypol: In mechanically pressed cottonseed meals in which free gossypol is low, the "bound" gossypol is a major damaging factor.

2. Free gossypol: In those cottonseed meals which have a high free gossypol content, the free gossypol may be a major damaging factor.

B. Nitrogen Solubility

1. For intermediate levels of nitrogen solubility, there was no direct relationship between this criterion and nutritive value.

2. No conclusive evidence was presented to justify a change in the previous recommendation that cottonseed meals having 0.04% or less of free gossypol and 75% or higher nitrogen solubility in 0.02N NaOH solution are satisfactory for use in a 50:50 ratio with soybean meal in chick rations. (See also report on poultry.)

III. Cottonseed Meal in Poultry Rations

The Committee reaffirmed the validity of the statement on cottonseed meal made at the Third Conference on Cottonseed Processing and Nutritive Value of the Meal insofar as it refers to poultry. The statement reads as follows:

> "Results presented thus far indicate that chick and broiler rations containing cottonseed meal and soybean meal in equal proportions on a nitrogen basis are equal or superior to rations based on either cottonseed meal or soybean alone, when the cottonseed meal used has 0.04% or less of free gossypol and 75% or more of nitrogen solubility in 0.02N NaOH solution."

There is evidence that there exist cottonseed meals of 0.04% free gossypol content but with less than 75% nitrogen solubility, which when substituted for 50% of the soybean meal in a practical broiler ration, give excellent performance. The committee feels, however, that it should be the objective of cottonseed processors when preparing meals for broiler rations, to retain the maximum quantity of desirable nutrients insofar as possible. It is the general experience that with a given process, higher nitrogen solubility generally reflects less damage to or less destruction of desirable nutrients.

IV. Cottonseed Meal in Swine Rations

Available data indicate that a dietary level of 0.0045% free gossypol can be fed without evidence of toxicity. A level of 0.0066% is the lowest dietary level that has been reported to be toxic.

V. Cottonseed Meal in Ruminant Rations

1. Cottonseed meal can be fed as the only source of supplementary protein for growth and reproduction of ruminants with satisfactory results.

2. Recent growth and metabolism studies indicate that differences exist among cottonseed meals and other vegetable protein supplements as sources of protein for lambs.

VI. Impact of Research on the Cottonseed and Feed Industries

The information developed by this program has caused widespread re-evaluation of the nutritional properties of cottonseed meal by research workers, cottonseed processors, feed manufacturers, and feeders. It has resulted in their recognition of the important contribution which cottonseed meal can make to the poultry and swine industries.

Studies reported here emphasized again that processing conditions which employ minimum heat produce meal of highest nutritive value, provided the free gossypol content is reduced below the tolerance level of the animal fed.

VII. Research Recommended by Committee

A. Chemical Research

1. Expanded work on the reactions of gossypol.

2. Research to characterize the different compounds measured as total and/or free gossypol.

3. Research on lysine:

a. Continued studies on the relationship between the lysine content and the nutritive value of cottonseed meals.

b. Similar studies on the possibility of using the determination of free epsilon amino groups of lysine as a measure of the nutritive value of cottonseed meals.

c. Investigations to develop a simplified method for lysine determination.

4. Research on the egg discoloration problem:

As a result of fundamental investigations on the chemistry and structure of gossypol, there is now a clearer understanding of the nature of the materials that are responsible for egg yolk discoloration. Moreover, there are now available several chemical approaches for removing this material from cottonseed meal. Investigations of these approaches as well as approaches to the egg white discoloration problem should be pursued vigorously.

5. Research on the reactions of isolated cottonseed proteins with other constituents of the meal such as gossypol, carbohydrates and phosphorus compounds.

B. Processing Research

1. An extension of investigations to determine the effect of processing on amino acids.

2. An investigation of processing conditions needed to yield meals low in free gossypol, in which the formation of bound gossypol is at a minimum, and which retain maximum lysine content.

C. Nutritional Research

1. Cottonseed meals representative of the best production should be made available to research investigators.

2. Study the effect of bound and free gossypol on the nutritive value of cottonseed meals.

3. Conduct further work to determine whether differences exist among cottonseed meals as source of protein for calves, beef cattle, and dairy cattle.

4. Continue study of digestibility of protein, as well as other possible factors affecting use of cottonseed meal for lambs.

5. Study of the use of cottonseed meals in swine breeding type rations.

6. Determine nutritive quality of cottonseed meals containing 50% protein.

OPENING REMARKS

WELCOMING ADDRESS

C. H. Fisher, Director
Southern Utilization Research and Development Division
New Orleans, Louisiana

It has been our privilege to be host to the previous conferences on relation of cottonseed meal processing to meal nutritive quality and to this 1957 conference. It is a pleasure and a distinct honor to have you, the principal research leaders and planners in the field of processing and nutrition, in our Laboratory. There is a feeling of optimism about the future of cottonseed meal because of the magnitude of effort being expended in research. The caliber of the research is high, and the coordination and cooperation are excellent.

Our laboratory in New Orleans has done both applied and fundamental research toward bettering the utilization of cottonseed meal. Some work has been done under contract. For example, under contract work, Dr. Shirley at the University of Tennessee has proved that the structure hypothesized for gossypol by Roger Adams and associates in the 1930's is correct. With the fundamental information from his work, practical results can be expected toward solving many of the gossypol problems involved in processing cottonseed meal and oil.

We are grateful and proud of the privilege of being host to this conference.

RESPONSE

R. Stokes
The Buckeye Cellulose Corporation
Cincinnati, Ohio

I wish to extend the appreciation of myself and other members attending this meeting for this cooperative effort. I think conferences of this nature are a wise investment of public funds, and the results to be disclosed at this meeting will be very important in the future to the cottonseed industry. This conference can be and will be important in forming our conclusions about the utilization of cottonseed meal. The results to be disclosed by you, the personnel from various industrial, university, and governmental research laboratories, should be and will be seriously considered when formulating recommendations for future work on cottonseed meal and oil.

We wish to extend our appreciation to this laboratory for allowing us to meet here and for arranging this program. The representatives of various industrial firms wish each of you research workers success in your endeavors and the pleasure and well-being that stem from the feeling of accomplishments from a job well done.

THE COTTONSEED CRUSHING INDUSTRY'S INTEREST IN RESEARCH

A. L. Ward, Director
Educational Service
National Cottonseed Products Association, Inc.
(Presented in absentia)

This conference has, from the beginning, been a great stimulus and encouragement to me personally and to the cottonseed crushing industry; and I am truly indebted and grateful to each of you, personally, and to each of the states you officially represent.

I desire to express to Dr. Harold Wilcke of the Research Division of Ralston Purina Company our sincere appreciation of his generosity in giving so freely of his time and of his untiring efforts as Chairman of the Research Committee of the National Cottonseed Products Association. I desire also to express our appreciation to Dr. Fisher and to Dr. Altschul. Dr. Altschul and his staff, whose work relates specifically to the nature of this conference, have, for many years, served as a clearing house on research important to cottonseed processors and to the livestock and poultry producers of our Nation.

It is an established fact that the ability of manufacturers or processors of any commodity to stay in business hinges on discovering and making use of techniques that aid in the development of more efficient and profitable methods of processing the quality products demanded by users of their products. This fact is the foundation of the cottonseed crushers' interest in research.

Research projects now being supported by the National Cottonseed Products Association have the goal of discovering techniques which we hope will aid in developing more efficient and profitable methods of processing cottonseed meal and oil that possess the quality demanded by users of these products.

It takes time for any industry to establish the fact of improved quality of products and where there are many processors in a given industry, such as the cottonseed crushing industry, uniformity of quality products is acquired more slowly than is possible under single control management.

In spite of the difficulty of many managements there is evidence in the market place that the trade in protein is seeking cottonseed meal because of some of its improved qualities and in the near future we hope to show improvement of our already high-quality cottonseed oil.

The serious interest of the cottonseed crushing industry in research is evidenced by the fact that crushers, through the Southern Regional Research Laboratory and through land-grant colleges and universities, have, over a period of many years, continued to lend encouragement and, in many instances, financial assistance to both fundamental and applied research important not only to the processors of cottonseed but to the livestock and poultry producers of our Nation.

The cottonseed crushers are alert to the fact that, through coordinated and diligent efforts, we must hasten the day when we can take from research laboratories findings and ideas and apply them to processing at our cottonseed crushing mills and cotton oil refining plants.

Our Research Committee of the National Cottonseed Products Association is grateful for the great strides that have been made, but we need to have more and more facts and information. Therefore, the cottonseed processors and oil refiners welcome the assistance and the cooperation of research workers such as you. I know of no group of scientific workers more capable and more devoted to the cause of science and nutritional research, and I wish for each of you success and great satisfaction.

TRENDS IN THE USE OF COTTONSEED MEAL FOR LIVESTOCK IN THE SOUTH*

C. W. Upp, Director
Louisiana Agricultural Experiment Station
Baton Rouge, Louisiana

There have been rapid changes in agriculture in the South and particularly in Louisiana. Today more than 93 percent of the farms in Louisiana have electricity. This rapid change has been seen in the growth of the livestock industry in the South. This naturally has a direct bearing on the utilization of cottonseed meal for livestock feeding. If a high quality cottonseed meal for animal feeding can be produced, the South should be able to assimilate the total production of meal for feeding of livestock within its areas.

The byproduct, cottonseed meal, and its utilization are influenced greatly by economics. The meal will be in a more advantageous position when the efficiency of meal production has been increased to warrant a lowering in the price of the final product. Cottonseed meal will be in better position to compete with other protein supplements on an economic basis for use in livestock rations. Of course, the problem of economics is not limited only to the meal processing conditions. It is related to that of cotton itself. The exports of cotton have decreased. In 1955 the quantity of cotton exported dropped to 29 percent of the world trade value for cotton. Moreover, there is constant competition from manmade fibers. Within the last thirty years there has been a constant decrease in the amount of cotton fiber employed commercially and a concurrent increase in the amount of synthetic fiber employed.

Through constant research efforts to find most efficient methods for production of cottonseed meals of high nutritive quality, the utilization of cottonseed meal will be assured. For example, the number of animal units of livestock in seven Southern states, excluding Oklahoma and Texas, has increased from 12 to 15 million units within the last thirty years. Without feeding a pound of cottonseed meal to farm animals other than swine and poultry, all of the cottonseed meal produced could be utilized by the animal feeding industry of the Southern States.

Since the ultimate consumption of cottonseed meal is dependent upon the ability of the meals to compete price-wise and quality-wise with other oilseed meals, the only solution to the problem is to find the most efficient means for production of the best-quality cottonseed meal possible. The cottonseed meal processors then will be able to sell their entire meal production within their own areas.

* Presented at a luncheon held January 16, 1957.

CURRENT COTTONSEED RESEARCH PROGRAM

H. L. Wilcke, Chairman of Research Committee
National Cottonseed Products Association
Dallas, Texas

Several years ago I was asked to serve on the Research Committee of the National Cottonseed Products Association for the period of one year, and one year only. Here it is, three years later, and I find I am still actively and willingly serving and learning on the Committee.

In my work with the Committee, I find myself respecting the work and the attitude of the group, which has been directed strictly and intensely on research. The object of the Committee has been to obtain fundamental information on cottonseed products and byproducts and to make use of this information in producing the highest quality products and in promoting a sound increase in the use of these products.

More specifically, the goal has been to encourage research on cottonseed products in experimental laboratories, governmental laboratories, industrial laboratories and any other capable research institution. This has been successfully achieved through the efforts and work on the part of A. L. Ward's group, through grants-in-aid, governmental agencies such as the Southern Regional Research Laboratory and through the moral support of industry.

Work has been encouraged in view of the existing needs both on oils and on meals. With regard to oil: Processing, refining and utilization of oil has been accomplished through improved production. With respect to cottonseed meal and other oilseed meals, including sesame: Markets for such type seed meals have been developed, improved and broadened for inclusion in livestock and poultry rations. There exists, always, an interest in finding more economical, better performing protein supplements.

We have supported work in crop improvement and have produced higher yielding cottonseed, which is low in gossypol and high in protein efficiency. There exists excellent cooperation on programs between the people in industry and the field of education.

Such conferences as this one and the ones that preceded this, serve to promote work on a highly cooperative, coordinated plane, whereby results and advice are obtained which are invaluable in showing up those lines of work which need financial support and guidance, and further, indicate what must be done in the future.

We have entered into the field of nutrition of cattle, sheep, swine, and poultry; we are also in the field of processing. A committee was appointed on cottonseed meal products and another committee on the processing of oil. The Research Committee, comprised of Gunn, Hickey, Johnson, Robinson, Stokes, Williams, Wilcke, Ward, Harper and Moloney, asked for four committees to observe, analyze and formulate recommendations on the information to be presented at this conference.

The committee members on chicks and the use of cottonseed meal in chick rations are A. M. Altschul, chairman, and J. Corbin, C. A. Denton, J. M. Dechary, H. D. Fincher, C. R. Grau, R. Stokes, J. Waddell, A. B. Watts, W. S. Wilkinson.

The committee members on swine and ruminants are A. A. Heidebrecht, chairman, and I. P. Earle, E. R. Barrick, L. V. Curtin, K. T. Holley.

The committee members on chemical measures are C. M. Lyman, chairman, and V. L. Frampton, H. E. Robinson, C. L. Hoffpauir, P. D. Aines, H. L. Wilcke.

The committee members on the relationship of the research program to industry are G. A. Harper, chairman, and H. J. Konen, P. A. Williams, G. E. Goheen, J. F. Moloney, C. W. Upp.

SUMMARY OF HISTORY OF COTTONSEED MEAL COLLABORATIVE CONFERENCES

A. M. Altschul
Southern Regional Research Laboratory
New Orleans, Louisiana

The Educational Service of the National Cottonseed Products Association and various research groups re-appraised the status of cottonseed meals in non-ruminant feeds in 1949. Research studies were made on cottonseed meals and the results were reported in collaborative conferences held in 1950, 1951, and 1953 at the Southern Utilization Research and Development Division, New Orleans, Louisiana. After the 1953 conference, it was possible to make definite statements about the use of cottonseed meals in poultry rations. We delayed the Fourth Conference until this year so that new data could be collected and lengthy feeding studies be made with commercially processed cottonseed meals.

The purposes of this conference are manyfold, but our two main purposes are: (1) to take stock of the present knowledge from this recent study in order to make definite statements helpful to processors and feed manufacturers for production of high quality cottonseed meals, and (2) to decide on future lines of research. This conference also provides an informal means for each of you to exchange information and to become reacquainted. With the results to be reported at this conference, we can feel that we have made much progress toward our ultimate goal -- the unrestricted use of cottonseed meals in swine and poultry rations.

We regret deeply that due to illness, Mr. A. L. Ward was unable to attend this conference. You, who work with Mr. Ward, please carry our good wishes for a speedy recovery.

Seminar I - REPORT ON COLLABORATIVE STUDY OF COMMERCIALLY PROCESSED COTTONSEED MEALS

Chairman - A. M. Altschul, Southern Regional Research Laboratory, New Orleans, Louisiana

INTRODUCTION TO COLLABORATIVE STUDY

A. M. Altschul
Southern Regional Research Laboratory
New Orleans, Louisiana

At this time I wish to read the statements made at the third conference on relation of processing to nutritive quality of cottonseed meals. Those statements were: "Results presented thus far indicate that chick and broiler rations containing cottonseed meal and soybean meal in equal proportions on a nitrogen basis are equal or superior to rations based on either cottonseed meal or soybean alone, when the cottonseed meal used has a 0.04% or less of free gossypol and 75% or more of nitrogen solubility in 0.02N sodium hydroxide solution.

Preliminary indications are, insofar as free gossypol is concerned , that cottonseed meals having 0.04% or less of free gossypol can be fed in unrestricted proportion in balanced diets for chicks, broilers and swine."

It was on the basis of those statements that we undertook this large scale cooperative study being reported today. Some investigators have told me that this present study represents the largest nutrition experiment undertaken. I don't know whether we can make such a statement. I should like to express the purpose of this experiment. It was to determine the use of mixtures of commercial cottonseed meals and soybean meal in practical poultry rations. We screened samples of cottonseed meals prepared by various processes from many mills throughout the country. From the range of properties exhibited by the meals in the screening test, we selected those which appeared to be most representative of each method of cottonseed processing. The selected meals were mixed in various proportions with soybean meal and added to a practical broiler basal ration. Samples of the rations and meals were distributed to federal and state experiment stations, universities and research laboratories of interested commercial firms. Mr. Piccolo will now give you a detailed description of ration preparations, their distribution for feeding, and Dr. V. L. Frampton will present some statistical analyses of the data.

PRESENTATION OF ANALYTICAL DATA ON COTTONSEED MEALS

B. Piccolo
National Cottonseed Products Association Fellow
Southern Regional Research Laboratory
New Orleans, Louisiana

At a meeting held at this Laboratory in November, 1955, it was decided that 8 cottonseed meals, representative of all commercial processing methods, would be selected for this collaborative study. Cottonseed oil mills throughout the country were contacted and requested to send one lb. samples of meal typical of their daily production to us for analysis. Some 50-odd samples of meals were received. These samples were subjected to 4 screening analytical procedures, involving analyses for free gossypol, total gossypol, total nitrogen and nitrogen soluble in 0.02N sodium hydroxide solution. A very wide variation was found in the constituents of meals prepared by different processing methods. Table I shows the range of values for the meals investigated. From the results of that screening test, 8 meals were chosen as being most representative of the types of meals produced by each cottonseed processing method employed today.

The mills producing the selected meals were contacted, and a portion of their daily production was set aside and forwarded to St. Louis, where the actual mixing of the rations took place in the Ralston Purina plant. Dr. Frampton and I were present when the meals were blended with a basal ration, cf. Table II. Each cottonseed meal was tested at three different levels in three different types of rations:

(1) Ration type No. 1; the cottonseed meal contributed 100% of the supplementary protein of the ration;

(2) Ration type No. 2; the protein supplement of the ration consisted of 75% cottonseed meal and 25% soybean meal on a nitrogen basis;

(3) Ration type No. 3; the protein supplement consisted of a 50:50 ratio of cottonseed meal to soybean meal on a nitrogen basis.

The 52% protein soybean meal was obtained from the American Soybean Association; this meal was also used as the reference ration (control ration) in which it contributed all of the protein supplement. More than 60 tons of poultry rations were mixed for the collaborative experiment. The compositions of the rations are shown in Table III.

Mixed rations were supplied to various collaborators for the actual chick feeding studies. The number of birds fed rations in this collaborative study are also shown in Table II. Samples of the cottonseed meals and soybean meal control were also supplied to other collaborators for studies with swine, rats, dairy and beef cattle, lambs and laying hens. One-pound samples of the selected meals and final rations were brought to this Laboratory where they were exhaustively analyzed for the following constituents: total nitrogen, nitrogen soluble in 0.02N sodium hydroxide solution, moisture, oil, iodine number of the oil, total phosphorus, inorganic phosphorus, total and reducing sugars, lysine, free gossypol and total gossypol. The results of these analyses are presented in Tables IV through VII.

In the following table are shown the cottonseed meals selected for feeding studies:

Cottonseed Meals Used in Collaborative Study	Description of Meals
CM-6	Prepress, solvent-extracted
CM-10	Prepress, solvent-extracted
CM-13	High speed screw-press
CM-16	Hydraulic-press
CM-19	Solvent-extracted
CM-21	Low speed screw-press
CM-36	Low speed screw-press
CM-45	Prepress, solvent-extracted
CM-49	Solvent-extracted, degossypolized

TABLE I

RANGE OF VALUES FOR VARIOUS CONSTITUENTS IN COMMERCIAL COTTONSEED MEALS

Type of Processing	Number of Meals Investigated	Free Gossypol %	Total Gossypol %	Total Nitrogen %	Nitrogen Soluble in 0.02N NaOH %
Low speed screw-press	14	0.02-0.09	0.70-1.41	5.9-7.3	35.79-62.52
High speed screw-press	10	0.02-0.08	0.75-1.38	5.5-6.9	27.91-40.91
Hydraulic-press	5	0.09-0.34	1.0-1.38	5.7-7.1	65.07-75.17
Solvent-extracted	2	0.24-0.40	1.06-1.08	6.6-6.7	69.19-82.79
Prepress, solvent-extracted	12	0.03-0.08	0.93-1.64	6.2-7.5	61.38-73.56

TABLE II

COMPOSITION OF BASAL RATION

	%
Ground yellow corn	50.00
Fish solubles	2.50
Dried whey (50% Lactose)	2.50
Dehy. alfalfa meal (17% protein)	2.00
dl-Methionine	0.05
Limestone, ground	1.60
Di-Calcium phosphate (20% P, 24% Ca.)	1.75
Sodium Chloride	0.50
Tallow (stabilized)	2.00
Total	62.90

NUMBER OF BIRDS FED RATIONS IN COLLABORATIVE STUDY

Ration Number	1	2	3	4	5	6	7	8	9	Total No. Birds Fed
CM-6-1	--	--	---	---	---	--	68	120	---	188
CM-6-2	--	--	---	---	---	--	70	118	---	188
CM-6-3	--	--	---	---	---	--	68	130	---	198
CM-10-1	78	28	118	113	107	81	--	---	113	638
CM-10-2	80	30	118	114	119	77	--	---	120	658
CM-10-3	59	29	120	119	120	77	--	---	80	604
CM-13-1	59	--	116	---	117	--	--	105	120	517
CM-13-2	79	--	118	---	119	--	--	119	117	552
CM-13-3	80	--	120	---	118	--	--	118	116	552
CM-16-1	--	32	120	---	---	--	--	---	---	152
CM-16-2	--	31	120	---	---	--	--	---	---	151
CM-16-3	--	30	120	---	---	--	--	---	---	150
CM-19-1	--	--	---	---	118	--	--	---	110	228
CM-19-2	--	--	---	---	120	--	--	---	113	233
CM-19-3	--	--	---	---	120	--	--	---	119	239
CM-21-1	--	--	120	---	114	--	--	119	120	473
CM-21-2	--	--	118	---	120	--	--	115	118	471
CM-21-3	--	--	120	---	119	--	--	119	119	477
CM-36-1	--	32	112	118	80	75	--	---	116	533
CM-36-2	--	--	118	120	120	79	--	---	79	516
CM-36-3	--	32	120	116	80	79	--	---	114	541
CM-45-1	80	30	120	---	118	--	--	---	117	465
CM-45-2	80	32	118	---	120	--	--	---	116	466
CM-45-3	80	32	120	---	118	--	--	---	116	466
CM-49-1	--	32	120	120	120	--	--	---	104	496
CM-49-2	--	--	118	119	120	--	--	---	117	474
CM-49-3	--	--	119	117	130	--	--	---	77	443
CM-50	79	124	120	130	120	80	60	119	116	948
									Total	12,017

TABLE III

COMPOSITION OF RATIONS

RATION NO.	50	6-1	6-2	6-3	10-1	10-2	10-3
Ingredients							
Basal	62.90	62.90	62.90	62.90	62.90	62.90	62.90
Soybean Meal	27.14	0.00	7.00	14.00	0.00	7.00	14.00
Trace Minerals	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Fat	0.72	2.07	2.01	1.96	1.93	1.91	1.89
Glucose	8.14	0.72	2.34	3.94	3.60	4.49	5.37
Cottonseed Meal	0.00	34.21	25.65	17.10	31.47	23.60	15.74

RATION NO.	13-1	13-2	13-3	16-1	16-2	16-3	19-1	19-2	19-3
Ingredients									
Basal	62.90	62.90	62.90	62.90	62.90	62.90	62.90	62.90	62.90
Soybean Meal	0.00	7.00	14.00	0.00	7.00	14.00	0.00	7.00	14.00
Trace Minerals	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Fat	0.95	1.17	1.40	0.00	0.46	0.92	1.46	1.70	1.75
Glucose	1.37	2.81	4.26	0.27	2.00	3.72	0.83	2.41	3.99
Cottonseed Meal	34.68	26.08	17.34	36.73	27.54	18.36	34.53	25.89	17.26

RATION NO.	21-1	21-2	21-3	36-1	36-2	36-3	45-1	45-2	45-3	49-1	49-2	49-3
Ingredients												
Basal	62.90	62.90	62.90	62.90	62.90	62.90	62.90	62.90	62.90	62.90	62.90	62.90
Soybean Meal	0.00	7.00	14.00	0.00	7.00	14.00	0.00	7.00	14.00	0.00	7.00	14.00
Trace Minerals	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Fat	1.51	1.59	1.68	1.80	1.81	1.83	1.97	1.94	1.91	1.39	1.50	1.62
Glucose	0.00	0.95	3.01	0.67	2.30	3.91	0.23	1.97	3.69	4.62	5.26	5.88
Cottonseed Meal	35.49	27.46	18.31	34.53	25.89	17.26	34.80	26.09	17.40	30.99	23.24	15.50

TABLE IV

TOTAL NITROGEN AND CRUDE FIBER CONTENTS OF RATIONS

Meal No.	Ration No. 1	Ration No. 2	Ration No. 3	Control Ration
	% Total Nitrogen Content			
6	3.52	3.36	3.46	
10	3.19	3.16	3.21	
13	3.42	3.48	3.56	
16	3.26	3.51	3.38	
19	3.41	3.59	3.63	
21	3.54	3.41	3.51	
36	3.48	3.75	3.60	
45	3.42	3.44	3.36	
49	3.39	3.55	3.49	
50				3.52
	% Crude Fiber Content			
6	6.36	5.29	4.34	
10	5.91	4.43	4.04	
13	6.11	4.81	4.18	
16	6.30	5.18	4.32	
19	5.59	4.68	3.99	
21	6.10	5.33	4.37	
36	5.54	4.52	3.85	
45	6.60	5.56	4.44	
49	4.28	3.31	3.84	
50				2.45

TABLE V

NITROGEN SOLUBILITY AND FAT CONTENT OF RATIONS

Meal No.	Ration No. 1	Ration No. 2	Ration No. 3	Control Ration
	% Nitrogen Soluble in 0.02N Sodium Hydroxide			
6	74.16	80.62	80.33	
10	60.00	68.04	70.19	
13	51.33	59.77	69.66	
16	71.41	72.45	76.77	
19	74.26	70.56	70.88	
21	56.56	65.87	71.02	
36	56.75	57.71	67.17	
45	72.29	74.36	80.01	
49	82.06	80.06	80.00	
				80.77
	% Fat Content			
6	6.67	6.61	6.62	
10	6.52	6.79	6.33	
13	6.39	6.49	6.28	
16	6.55	6.54	6.51	
19	6.48	6.46	6.48	
21	7.40	7.24	6.76	
36	7.27	6.96	6.81	
45	6.65	6.44	6.50	
49	6.45	6.55	6.56	
50				6.32

TABLE VI

IODINE NUMBERS AND MOISTURE CONTENTS OF RATIONS

Meal No.	Ration No. 1	Ration No. 2	Ration No. 3	Control Ration
		Iodine Number of Fat		
6	69.15	75.14	75.66	
10	74.65	75.81	76.97	
13	78.22	78.37	76.47	
16	90.89	87.76	82.71	
19	78.13	78.91	77.65	
21	82.38	81.96	77.08	
36	77.02	78.38	76.01	
45	75.27	75.80	73.94	
49	81.09	79.85	78.69	
50				62.80
		% Moisture		
6	10.11	10.06	9.88	
10	9.30	9.36	9.17	
13	10.02	10.70	10.61	
16	10.37	10.36	10.46	
19	10.21	10.57	10.42	
21	9.83	9.68	9.68	
36	9.85	10.49	10.33	
45	10.30	9.97	9.90	
49	11.06	10.91	10.78	
50				10.38

TABLE VII

CHEMICAL DATA ON COTTONSEED MEALS USED IN RATIONS

	CM-6	CM-10	CM-13	CM-16	CM-19	CM-21	CM-36	CM-45	CM-49
% Total gossypol & gossypol derivatives	0.75	1.00	1.00	1.11	0.96	0.62	1.10	0.75	0.84
% AOCS "free" gossypol and gossypol-like pigments	0.02	0.04	0.03	0.14	0.28	0.02	0.05	0.04	0.04
% Gossypol [a]	0.007	0.014	0.013	0.055	0.135	0.007	0.011	0.019	0.029
% Inorganic phosphorous	0.419	0.431	0.417	0.502	0.320	0.397	0.451	0.426	1.115
% Organic phosphorous	0.775	0.723	0.802	0.656	0.956	0.854	0.924	0.586	0.034
% Total Fat	0.17	0.64	3.40	5.80	1.40	1.74	0.94	0.45	2.38
Iodine No. of Fat	94.19	96.18	107.6	103.3	97.24	103.2	102.5	97.39	94.77
% Total sugar	6.73	7.09	6.33	5.56	7.07	6.09	7.72	6.58	7.44
% Reducing sugar	0.86	0.87	0.99	0.92	0.88	0.87	0.86	0.89	0.59
Grams lysine per 16 grams of nitrogen	4.00	3.42	3.44	3.64	3.87	3.87	3.61	3.86	--
% Crude Fiber	11.36	12.72	12.44	11.89	10.64	11.53	9.58	13.49	9.60
% Nitrogen	6.55	7.12	6.46	6.10	6.49	6.12	7.13	6.44	6.78
% Nitrogen soluble in 0.02N NaOH	68.74	52.40	30.10	65.09	70.00	46.76	39.10	65.90	83.66
% Moisture	9.32	7.90	7.02	8.78	10.73	9.15	3.25	8.65	--

[a] King, W. H., Frampton, V. L., and Altschul, A. M., J. Am. Oil Chemists' Soc., 34, 247-249 (1957).

INTERPRETATION OF RESULTS

V. L. Frampton
Southern Regional Research Laboratory
New Orleans, Louisiana

Commercial cottonseed meals vary widely in chemical properties such as protein solubility, gossypol, lysine or sugar content. This is true of cottonseed meals which are produced at different mills with the same type of equipment. This variation is reflected in the growth response of broilers fed on rations containing such cottonseed meals. With respect to the meals themselves, the correlation between protein solubility and total gossypol is very poor; the same is true for protein solubility and bound gossypol. The odds for significance of a correlation between bound gossypol and lysine content of the meals are 200:1, while the odds for significance of a correlation between protein solubility and lysine content of the meals are only 24:1.

The agreement in weights of chickens at 4 and at 8 weeks on a given ration fed at the several stations was very poor, while the agreement in the alignment of the several rations at each of the various stations was good.

The growth response of the chickens was found to be correlated negatively with the total gossypol and with the crude fiber content of the rations. The correlation of the growth response with lysine and with the energy of the rations was positive. A poor correlation was found between the "free" gossypol of the rations and growth response of the chickens. The solubility (in 0.02N sodium hydroxide solution) of the proteins in the rations is correlated with the growth response of the chickens.

The growth response of the chickens on most of the rations containing cottonseed meal was not as good as that shown on the control ration. A portion of the reduced growth response is probably attributable to the crude fiber (largely cottonseed hulls) of the rations containing the cottonseed meals, but the reduction attributable to crude fiber is small in comparison with that attributable to the total gossypol.

Discussion

Harper: Please define the soybean meal used.

Frampton: It was a blend of 4 commercial soybean meals, obtained with the help of the American Soybean Association. It contained 52% protein, which was higher than the protein content of any cottonseed meal used in this present study.

Aines: What was the nitrogen content of the soybean meal?

Frampton: It contained 52% protein. The control soybean meal ration contained 22% protein.

Wilcke: Why was glucose added to the rations?

Frampton: Since the cottonseed meals contained varying quantities of hulls, the nitrogen content of the meals varied. It was necessary to make suitable adjustments to give constant nitrogen contents in the rations. We were pushed for time and, since glucose was available, we used glucose. Of course, adding glucose to the rations introduced another variable.

Wilcke: Were the rations isocaloric?

Frampton: No. It was impossible to make them isocaloric because the cottonseed meals were not equivalent to the soybean meal control in protein and fiber contents.

Harper: Then you very definitely were measuring differences in fiber content?

Frampton: Yes. According to mathematical data, the fiber content of the rations was important.

Fincher: In calculation of regression correlation coefficients, have you included meals of high free gossypol content?

Frampton: Six out of 28 rations had high free gossypol. The results may not be conclusive enough to warrant predictions about the performance of animals fed high free gossypol content meals, however.

Eagle: Can you state that there is no correlation between free gossypol and weight gains?

Frampton: No. The correlation coefficient was not zero in the case of free gossypol versus growth, but it was small. It was also small in the case of inorganic phosphorus versus growth and of nitrogen solubility versus growth. We have completed only the basic correlations on the IBM machine, and analyses of data so far predict only trends. Total gossypol, lysine and crude fiber appear to be the more important factors. There were vast differences between stations, but there were good alignments within stations.

Lyman: Since every station did not test every ration, we should be cautious in interpretation of the data.

Frampton: We have calculated the data for one station which did test all rations. We plan to calculate data for every station, but we could not complete statistical analysis in time for this meeting.

Hundley: Are calculated values for stations having large or small colonies of chicks?

Frampton: These are in small colonies.

Stephenson: Is your calculation for fiber content valid due to interference from soybean meal in the ration?

Frampton: The correlation coefficient should give the true effect of fiber in the presence of soybean meal.

Stephenson: I think the good performance obtained on the 50:50 mixtures of cottonseed meal-soybean meal stresses the importance of amino acid constituents and not fiber contents.

Holley: What was the percent variation accounted for by your equation?

Frampton: I did not say what the variance was. The predicted weight in grams of males at 8 weeks, on the basis of the computations completed at this time, is 238 - 3.9 x % of hulls + 1,293 x % of lysine - 2,130 x % of total gossypol.

Watts: The amount of food intake per animal has to be considered.

Frampton: Yes. The calculated values would not only be dependent upon the nitrogen contents of the rations, but also upon food intake of the chicks. In the case of Watts' studies, there was a 1.0 correlation between food intake and growth. In other stations the correlation coefficient was 0.97 to 0.98. Since these values were close to unity, we can talk about weight gains in terms of rations fed.

Rusoff: Do you have the lysine content of the basal ration?

Frampton: It can be calculated as the difference between the lysine content of whole ration and that of protein source (cottonseed meal or soybean meal).

Lyman: What would you say is the most important factor involved?

Frampton: Total gossypol, since it exhibits the largest regression coefficient.

Heywang: But how can data be arranged to say that total gossypol is important and free gossypol is not important?

Frampton: The regression coefficient for effect of free gossypol on growth is 0.4; that for total gossypol is 0.8.

Lyman: Results from statistical analyses are in close agreement with those from other tests. There is a definite relationship between total gossypol and growth.

AMINO ACID SUPPLEMENTATION TO BROILER RATIONS

O. C. Bucek
The Dow Chemical Company
Lake Jackson, Texas

Feeding studies were made on White Plymouth Rock chicks using the control soybean meal ration and diets where varying amounts of the soybean meal were replaced by screw-press, pre-press solvent-extracted, and degossypolized cottonseed meal. Data obtained on supplementation of these diets with amino acids show that feed efficiency is increased when dl-lysine is added to rations containing cottonseed meals. In the rations containing 100% cottonseed meal as a protein source, 4 grams of dl-lysine.HCl per pound of ration gave the best results; in rations containing 75% cottonseed meal and 25% soybean meal, 2 grams per pound of ration were desirable, while in the 50:50 ration, 0.85 gram per pound of ration was best. In addition, feed efficiencies among the rations containing meals of various processing conditions were equalized by the supplementation. CM-10 either as 75% or 100% of the soybean meal of the ration resulted in a low feed efficiency of around 4, while CM-36 and CM-49 when used in all three of the ration types resulted in low feed efficiency values ranging from 2.5 to 2.7 before supplementation. After supplementation, the feed efficiency values of the rations in which CM-10 was added as 100% and 75% of the soybean meal was improved to around 2.5; all rations containing CM-36 gave feed efficiency values around 2.2, while those containing CM-49 gave values around 2.5. All of these values equaled and some were superior to the value for feed efficiency of the control ration where soybean meal was used without supplementation.

Ration No.	Grams of dl-lysine/lb. of ration	Average Feed Efficiencies on Rations Containing			
		CM-10	CM-36	CM-49	Soybean Meal
	g.				
#1 100% c/s meal	None	4.34	2.52	2.60	
	2.0				
	3.0				
	4.0	2.48	2.23	2.38	
	5.0	2.41	2.23		
#2 75% c/s meal: 25% soybean meal	None	4.15	2.51	2.71	
	1.0	3.27	2.26	2.71	
	2.0	2.67	2.16	2.52	
	3.0	2.61	2.10	2.52	
	4.0	2.52	2.21	2.38	
#3 50:50 c/s meal: soybean meal	None	2.80	2.48	2.63	
	0.70	2.85	2.47	2.55	
	0.85	2.41	2.37	2.52	
	1.00	2.86	2.33	2.47	
CONTROL	None				2.42

Discussion

Waddell: Dr. Bucek, are your calculations based upon the amount of dl-lysine-hydrochloride added to rations or upon the amount of available lysine?

Bucek: Data are reported in terms of dl-lysine-hydrochloride added.

Waddell: In dl-lysine only about 46% of it is available for useful assimilation in the chick.

STUDY OF COMMERCIAL COTTONSEED MEALS IN CHICK RATIONS

William L. Hunter, Van P. Entwistle, William C. Green
California Department of Agriculture
Sacramento, California

Day-old Single-Comb White Leghorn cockerel chicks were grown to two weeks of age on the diet listed in Table I. At the end of the pre-test period, the chicks were individually weighed and assigned to groups of twenty each, on a weight basis. The average weight of all groups was about the same at the beginning of the experimental period. After the initial weighing, the birds were placed on the Southern Regional Research Laboratory test diets and weighed every two days during the experimental period of ten days. Three groups were continued on the pre-test ration as controls.

Electrically heated battery brooders having raised wire floors were used for both the preliminary and experimental periods. Feed and water were provided ad libitum.

The results of this study on the twenty-two Southern Regional Research Laboratory diets and the three groups on our laboratory diet, are listed in Table II.

This test has been used extensively in the California Department of Agriculture for comparison of protein meals and commercial fryer feeds, because it affords an economy of time and materials. Greatest weight customarily is given to rate of growth (% gain/day) in interpretation of the data. Using percent gain per day as a criterion for evaluation, 6.0 to 6.4 is considered good and 6.5 or greater, is considered excellent growth.

On this basis, the seven rations containing different cottonseed meals at the lowest test level supported good growth with rations 21-3 and 49-3 giving the best results. Of the rations supplying the greatest proportion of cottonseed meal protein, 49-1 produced the best growth and approximated the results obtained with the soybean meal ration (50). Other rations containing the highest level of cottonseed meal followed in order of decreasing productivity; 6-1, 21-1, 36-1, 10-1, 19-1, and 13-1. The last three rations are considered to be inferior as were companion rations containing the intermediate level of cottonseed meal (10-2, 19-2, 13-2).

Feed efficiency and protein efficiency generally were in close correlation with the rate of gain.

TABLE I

PRE-TEST BROILER RATION

Ingredients	
Fish Meal, Herring (75% Protein)	5 %
Soybean Meal, Solvent Process (44% Protein)	15 %
Meat and Bone Meal (55% Protein)	5 %
Ground yellow corn	44 %
Ground wheat	15 %
Ground barley	5 %
Alfalfa meal, dehydrated	4 %
Dried brewers yeast	3 %
Ground limestone	1.5 %
Steamed bone meal	1 %
Iodized salt	0.5 %
Cottonseed oil	1.0 %
3-nitro-4-hydroxyphenylarsonic acid	0.055%
Vitamin A	4400 U.S.P. Units/kg.
Vitamin D	300 I.C. Units/kg.
Choline Chloride	1.1 gm./kg.
$MnSO_4 \cdot H_2O$	140 mg./kg.
Riboflavin	2 mg./kg.
Niacin	10 mg./kg.
Penicillin Diamine	8 mg./kg.

TABLE II

EVALUATION OF COMMERCIALLY PROCESSED COTTONSEED MEALS
CHICK GROWTH DATA

Ration Number	Average Gain gm.	% Gain per day 1/	Feed Efficiency 2/	Protein Efficiency 3/
6-1	99	5.6	2.56	1.86
6-2	106	5.9	2.41	1.98
6-3	118	6.4	2.16	2.20
10-1	69	4.3	3.30	1.44
10-2	100	5.7	2.53	1.89
10-3	116	6.2	2.33	2.04
13-1	50	3.2	4.16	1.15
13-2	91	5.3	2.74	1.74
13-3	119	6.4	2.25	2.12
19-1	58	3.8	3.50	1.36
19-2	82	4.9	2.83	1.68
19-3	110	5.9	2.32	2.06
21-1	92	5.2	2.64	1.80
21-2	117	6.3	2.35	2.03
21-3	127	6.7	2.07	2.30
36-1	83	4.9	3.00	1.59
36-2	107	5.9	2.38	2.00
36-3	104	5.8	2.51	1.90
49-1	112	6.1	2.38	2.00
49-2	116	6.3	2.30	2.07
49-3	124	6.6	2.05	2.32
50	122	6.5	2.09	2.28
1 4/	115	6.3	2.26	2.11
2 4/	113	6.2	2.28	2.09
3 4/	114	6.2	2.27	2.10

1/ % gain per day = $\frac{\text{average gain x 100}}{\text{average wt. x no. days}}$

2/ Feed Efficiency = Grams feed consumed / grams gained

3/ Protein Efficiency = Grams gained / grams protein consumed
Protein consumed calculated on the basis of a 21% protein ration

4/ Samples Nos. 1, 2 and 3 are the laboratory's practical broiler ration.

THE EFFECT OF PROCESSING ON NUTRITIVE VALUE OF COTTONSEED MEAL

E. L. Stephenson
University of Arkansas
Agricultural Experiment Station
Fayetteville, Arkansas

Data obtained in chick feeding trials (4 weeks in duration) at the University of Arkansas indicated that, when the level of free gossypol was lower than 0.04%, protein solubility in 0.02N NaOH was an acceptable index of protein quality as expressed by growth rate. This observation was based on information obtained when cottonseed meal samples Nos. 6, 13, 21, and 49 were fed at levels calculated to supply 100% of the protein supplement of the diets. Samples Nos. 45 and 10 were unavailable for feeding at this level and were not used. In no case did the weights of chicks receiving cottonseed meal equal that of the control (NRC Standard Diet).

Additional information relative to protein quality has been obtained with cottonseed meals possessing various nitrogen solubility values (solubility in 0.02N NaOH). These data indicated that the principal limiting amino acid in cottonseed meal was lysine and that this deficiency increased as heat damage to the protein reduced nitrogen solubility. The addition of lysine (and in some cases methionine) restored optimum nutritive value for chicks. Corn-cottonseed meal diets when properly supplemented produced chick growth equal to that obtained when corn-soybean meal diets were fed.

NUTRITIVE VALUE OF COMMERCIAL COTTONSEED MEALS

P. D. Aines
Buckeye Cellulose Corporation
Cincinnati, Ohio

This project was one of a series of experiments conducted at various industrial, state, and federal laboratories to ascertain the nutritive value of cottonseed meals processed by several commercial methods. Seven (7) cottonseed meals and a high protein soybean meal (CM-50) were fed in practical-type rations to broilers. The following cottonseed meals were evaluated: 2 low speed screw-press meals (CM-21, CM-36), 1 high speed screw-press meal (CM-13), 2 pre-press solvent meals (CM-10, CM-45), 1 straight solvent-extracted meal (CM-19), and 1 chemically-treated, degossypolized, solvent-extracted meal (CM-49). Each of these meals was fed so as to replace 50, 75 and 100 percent (nitrogen basis) of the soybean meal in the basal ration.

The basal ration was slightly modified from the proposed standard broiler ration of the Animal Nutrition Research Council. The calorie/protein ratios of the rations were not equalized. Each ration was fed in 3 repetitions to groups of 10 male and 10 female Arbor Acre White Rock chicks. The birds were raised in batteries to 8 weeks of age. There was a general decrease in both weight gains and feed efficiencies in all groups from one repetition to another. This decline was probably due to increasingly warm weather. However, some deterioration of the rations may have occurred on storage.

Under the conditions of this experiment, the following observations are warranted: Chemically-treated, degossypolized, solvent-extracted meal was superior to all other cottonseed meals tested as the principal source of protein in broiler rations. The chemically-treated cottonseed meal, one of the low speed screw-press meals (CM-21), and one of the pre-press solvent meals (CM-45) were similar in feeding value when used to surplant 75 percent of the soybean meal. However, the other meals made by the low speed screw-press and pre-press solvent processes were appreciably lower in nutritive value than their counterparts. This result indicated considerable variation in these methods of processing. The high speed screw-press meal was inferior to the other meals when used to replace 75 or 100 percent of the soybean meal in broiler rations. The response of the chicks to rations containing the lowest level of the cottonseed meals was similar for each meal except straight solvent-extracted meal. The performance of the birds fed this meal was poor even at the 50 percent level of substitution. The high protein soybean meal surpassed all cottonseed meals in feeding value. However, the higher energy content of the basal ration containing soybean meal favors increased gain and feed efficiency by the birds on this ration. Hence, a direct comparison of the performance of the chicks on this ration with that of the chicks on the cottonseed meal rations is biased in favor of the higher energy ration. This relationship may also account for a part of the difference in performance of chicks fed different levels of the same cottonseed meal. The correlation between nitrogen solubilities and relative nutritive values of different cottonseed meals was only slight in this experiment.

Discussion

Corbin: Would you say that nitrogen solubility is a good measure of nutritive value?

Aines: At high and low levels of nitrogen solubility, there is good correlation with growth responses. But at intermediate levels (37% - 62% nitrogen solubility for meals in this experiment) it is not a measure of nutritive value.

COMMERCIAL COTTONSEED MEALS IN CHICK BROILER RATIONS

A. Heidebrecht
Western Cottonoil Company
Abilene, Texas

Three experiments were conducted during a period from April 17th to August 31st. The following diets were included in each of the three experiments: 50; 10-1, 10-2; 13-1, 13-2, 13-3; 19-1, 19-2, 19-3; 21-1, 21-2, 21-3; 36-1, 36-3; 45-1, 45-2, 45-3; 49-1, 49-2. Diets Nos. 1-3 and 36-2 were only included in Tests 2 and 3 and No. 49-3 was included only in Tests 1 and 2.

Twenty (10 male and 10 female) Vantress-White broiler type chicks were placed on each diet in each experiment. Feed consumption data and individual weights were obtained at 4 and 8 weeks of age.

Based on the average growth rate of chicks on each diet in all experiments at 8 weeks the diets could be ranked as follows: 50; 49-3; 45-3; 21-3; 36-3; 13-3; 49-2; 21-2; 45-2; 10-3; 19-3; 49-1; 36-2; 19-2; 45-1; 10-2; 21-1; 13-2; 19-1; 36-1; 10-1; 13-1. The average weights ranged from 1169 grams down to 344 grams. In efficiency of feed conversion, the diets could be ranked as follows: 50; 45-3; 21-3; 36-3; 49-3; 10-3; 19-3; 21-2; 36-2; 13-3; 49-2; 49-1; 45-2; 45-1; 19-2; 10-2; 13-2; 21-1; 36-1; 19-1; 10-1; 13-1. The feed required per unit of gain ranged from 2.03 to 3.30.

It was observed that the females fed the #1 and 2 series of diets were approximately as heavy and in many cases heavier than the males, thus indicating a more pronounced adverse affect of high levels of cottonseed meal on the performance of the male. Although growth was adversely effected by diets containing high levels of cottonseed meal, mortality did not appear to be associated with these diets. Weight gains in general were progressively reduced on all diets with each subsequent experiment. This probably can be attributed to the extremely high temperature which existed during Tests 2 and 3. Furthermore, the feed appeared to become less palatable with age.

EFFECT OF PROCESSING AND DIETARY LEVEL OF COTTONSEED MEALS ON CHICK PERFORMANCE

R. J. Lillie and C. A. Denton
Animal Husbandry Research Division, Agricultural Research Service
Beltsville, Maryland

Three cottonseed meals (CM-10, -13, and -45) prepared under the supervision of the Southern Regional Research Laboratory were tested in two trials with sex-linked chicks from New Hampshire males x Silver Cornish females. There were duplicate groups in each experiment with 20 chicks per group, 10 males and 10 females. Each of the meals was fed at levels to replace 100, 75 and 50% (on a nitrogen basis) of the soybean meal in the ANRC standard broiler diet. The ANRC diet was used as the control. Very poor growth and feed conversion were obtained when CM-10 and CM-13 replaced all of the soybean meal. The large percentage of chickens with abnormal feather pigmentation indicated a rather severe lysine deficiency. CM-45 meal supported a growth rate about double that of the other two meals, although some lysine deficiency was indicated. When cottonseed meal replaced only 75% of the soybean meal, growth and feed conversion were somewhat improved; a slight lysine deficiency was indicated, particularly CM-13 and again CM-45 gave the best performance. When cottonseed meal replaced 50% soybean meal a further improvement in growth was obtained with all the meals, with the performance of CM-45 approaching that of the control diet. The results of these trials indicate that 50 percent of the soybean meal may be replaced with a good grade cottonseed meal with but little impairment of the nutritional value of the diet.

COTTONSEED MEAL NUTRITIVE QUALITY

H. R. Halloran*, B. Maxwell and J. B. Lyle
Poultry Producers of Central California
Petaluma, California

Procedure:

One set of rations with a single source of cottonseed meal (diets 6-1, 6-2, and 6-3) was compared with the control ration. Sexed Vantress Cornish-New Hampshire Cross day-old chicks were used, six males and six females in each group. The indicated rations were fed in triplicate. Individual chick weights were taken at 4 and 8 weeks; feed conversions were determined.

Results:

With a single source of cottonseed meal:

(1) The average chick weights at 4 and 8 weeks decreased with successive increases in the percentages of cottonseed meal, cf. Tables I and II.

(2) The feed conversion (lbs. of feed per lb. of bird) increased with successive increases in the percentages of cottonseed meal, cf. Tables I and II.

* Present address: Feed Consultant, Petaluma, California.

TABLE I

Group No.	Diet No.	Diet	4 weeks Average Weight	4 weeks Feed Conversion*
695	50	Control	528	1.53
701	50	Control	505	1.59
707	50	Control	522	1.58
		Average:	518	1.57
696	6-1	No Soybean Meal, Pre-press Solv., Calif. Cottonseed Meal	317	2.00
702	6-1	" " " "	297	1.95
710	6-1	" " " "	327	1.94
		Average:	313	1.96
697	6-2	25% Soybean Meal, 75% Cottonseed Meal	445	1.81
705	6-2	" " "	415	1.82
711	6-2	" " "	452	1.76
		Average:	437	1.80
700	6-3	50% Soybean Meal, 50% Cottonseed Meal	507	1.68
706	6-3	" " "	485	1.68
712	6-3	" " "	521	1.66
		Average:	504	1.67

* Lbs. of feed per lb. of bird.

TABLE II

Group No.	Diet No.	Diet	8 weeks Average (Weight)	Feed Conversion*	Perosis	Mortality
695	50	Control	1451	2.03	1	1
701	50	Control	1414	2.05	1	0
707	50	Control	1430	2.01	0	0
		Average:	1432	2.03		
696	6-1	No Soybean Meal, Pre-press Solv.,Calif. Cottonseed Meal	1014	2.39	1	1
702	6-1	" " " "	967	2.39	1	1
710	6-1	" " " "	1038	2.27	0	0
		Average:	1006	2.35		
697	6-2	25% Soybean Meal, 75% Cottonseed Meal	1335	2.26	0	1
705	6-2	" " "	1183	2.21	2	0
711	6-2	" " "	1219	2.21	1	0
		Average:	1246	2.23		
700	6-3	50% Soybean Meal, 50% Cottonseed Meal	1340	2.13	1	0
706	6-3	" " "	1335	2.13	0	1
712	6-3	" " "	1377	2.09	1	1
		Average:	1351	2.12		

* Lbs. of feed per lb. of bird.

NUTRITIVE QUALITY OF COTTONSEED MEAL RATIONS FOR CHICKS

J. E. Corbin and H. L. Wilcke
Ralston Purina Company
St. Louis, Mo.

Three cottonseed meal samples (CM-6, CM-13, CM-21) were fed to chicks for 8 weeks with each cottonseed meal replacing 50%, 75%, and 100% of the soybean portion of the rations. The control ration contained soybean meal, a blend from four sources.

Results:

	Protein Source				
	Soybean Meal	Cottonseed Meal	Chick Weights 4 wks.	8 wks.	Lbs. Feed/ Lb. Gain
Control	100	--	368	1179	2.03
CM-6-1	--	100	214	794	2.48
CM-6-2	25	75	288	999	2.17
CM-6-3	50	50	345	1090	2.09
CM-13-1	--	100	104	306	3.89
CM-13-2	25	75	182	632	2.72
CM-13-3	50	50	300	1037	2.21
CM-21-1	--	100	176	700	2.59
CM-21-2	25	75	292	1013	2.16
CM-21-3	50	50	325	1088	2.07

In this experiment, cottonseed meal, replacing soybean meal as a source of supplemental protein, decreased gains significantly when added as 100%, 75%, or 50% of the supplemental protein of the ration. The pounds of feed required to produce a pound of gain increased as the percentage of cottonseed meal in the ration increased.

COTTONSEED MEAL-HULL BRAN STUDIES

A. B. Watts and C. M. Sasser
Poultry Industry Department
Louisiana State University and A and M College
Baton Rouge, Louisiana

Three growth studies have been conducted to investigate the effect of the dilution of high protein cottonseed meals with varying amounts of hull bran. The cottonseed meals were secured from the Buckeye Cotton Oil Company and the Western Cottonoil Company of Abilene, Texas. They contained 50.0 and 51.6% crude protein respectively. In the first study the Buckeye meal was diluted with 3, 6, 9 and 12% hull bran (see Table I). The results are presented in Table II. It will be noted that there were no significant differences among the lots regarding 8 weeks gain or feed efficiency.

In the second experiment the meal from the Western Cottonoil Company was used. This was diluted with 6.25, 12.5, 18.75 and 26.0% hull bran. The rations fed appear in Table III. A summary of the results of this study appear in Table IV. It will be noted that the soybean meal ration yielded significantly superior results to the cottonseed meal rations in both 8 weeks weights and feed efficiencies. The cottonseed meal rations did not differ among themselves as to average weight at 8 weeks. The ration containing the cottonseed meal diluted with 26% hull bran (40.3% protein) was significantly inferior to the other cottonseed meal rations in feed efficiency. The dilution of the cottonseed meal with 6.25, 12.5 and 18.75 percent hull bran did not significantly affect the eight weeks weight or feed efficiency.

The third study was conducted to determine the effect of the addition of 2% vegetable oil in counteracting the effect of the hull bran dilutions. The meal used was a blend of equal parts of the Buckeye and the Western Cottonoil meals. The hull bran additions were made in a similar manner to those in the second experiment. However, the chemical analyses of the resulting mixtures indicated that the meal-hull bran mixtures were more nearly like those of the first study. The data are presented in Table IV. In this study the soybean meal ration did not prove to be superior in either 8 weeks weight or feed efficiency. It will be noted that the feed efficiencies of the birds on the ration containing the highest level of hull bran tended to be higher but were not significantly different from those of the birds on the rest of the rations.

TABLE I

RATIONS FOR FIRST COTTONSEED MEAL HULL BRAN STUDY

Ingredients	1	2	3	4	5	6
Yellow Corn Meal	60.5	63.3	62.0	60.5	58.7	56.7
Soybean Meal (46.4%)*	25.0					
100% Cottonseed Meal**-0 hull bran(51.6%)		22.2				
97% Cottonseed Meal - 3% hull bran(50.0%)			23.5			
94% Cottonseed Meal - 6% hull bran(48.7%)				25.0		
91% Cottonseed Meal - 9% hull bran(47.8%)					26.8	
88% Cottonseed Meal -12% hull bran(45.3%)						28.8
Fish Meal (60%)	3.0	3.0	3.0	3.0	3.0	3.0
Alfalfa Leaf Meal (17%)	3.0	3.0	3.0	3.0	3.0	3.0
Steamed Bone Meal	2.0	2.0	2.0	2.0	2.0	2.0
Oyster Shell Flour	1.0	1.0	1.0	1.0	1.0	1.0
Salt	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin Premix***	2.0	2.0	2.0	2.0	2.0	2.0
Dried Whey	3.0	3.0	3.0	3.0	3.0	3.0

* Figures in Parentheses () represent actual protein analyses of the meals and hull bran mixtures.

** The cottonseed meal used was a 52% protein cottonseed meal obtained from Buckeye Cotton Oil Company.

*** Vitamin premix contained the following ingredients per pound: 0.015 lb. methionine, 0.01 lb. delsterol, 0.05 lb. penicillin supplement, 0.125 lb. cod liver oil, 0.025 lb. choline supplement, 0.025 vitamin B_{12} supplement and 0.05 lb. Forbee supplement.

TABLE II

SUMMARY OF RESULTS OF FIRST EIGHT WEEKS GROWTH TRIAL
WITH COTTONSEED MEAL HULL BRAN MIXTURES

Ration	Lot	Gain	Feed Efficiency
Soybean Meal	9	2.00	2.60
	12	1.81	2.40
100% Cottonseed Meal	5	1.90	2.50
	8	1.93	2.40
97% Cottonseed Meal 3% Hull Bran	2	1.73	2.60
	6	1.72	2.50
94% Cottonseed Meal 6% Hull Bran	1	1.88	2.40
	7	2.00	2.40
91% Cottonseed Meal 9% Hull Bran	4	1.78	2.50
	10	1.71	2.60
88% Cottonseed Meal 12% Hull Bran	3	1.99	2.40
	11	1.89	2.50

TABLE III

RATIONS USED IN SECOND COTTONSEED MEAL-HULL BRAN EXPERIMENT

Ingredients	Rations					
	1	2	3	4	5	6
Yellow corn meal	60.5	63.3	62.0	60.5	58.7	56.7
Soybean meal (46.4% protein)	25.0					
CSM (50.7% protein)		22.2				
CSM (47.5% protein)			23.5			
CSM (45.0% protein)				25.0		
CSM (42.3% protein)					26.8	
CSM (40.3% protein)						28.8
Fish meal	3.0	3.0	3.0	3.0	3.0	3.0
Alfalfa leaf meal	3.0	3.0	3.0	3.0	3.0	3.0
Steamed bone meal	2.0	2.0	2.0	2.0	2.0	2.0
Oyster shell flour	1.0	1.0	1.0	1.0	1.0	1.0
Salt	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix	2.0	2.0	2.0	2.0	2.0	2.0
Dried Whey	3.0	3.0	3.0	3.0	3.0	3.0
Crude protein	21.4	21.3	20.8	20.5	20.8	20.4
Crude fiber	3.2	3.3	3.8	4.2	4.8	5.2

Vitamin premix supplies per ton:
4 grams riboflavin, 8 grams pantothenic acid, 18 grams niacin, 5,300,000 Units vitamin A, 30,000 AOAC chick units vitamin D_3, 4 grams procaine penicillin, 6.25 milligrams vitamin B_{12}, 0.50 pounds DL-methionine.

SUMMARY OF RESULTS OF SECOND COTTONSEED MEAL-HULL BRAN EXPERIMENT

Ration	Average Weight	Feed Efficiency
	8 weeks	8 weeks
Soybean meal I 46.4% protein	2.51 2.50	2.38 2.48
Cottonseed meal II 50.7% protein	1.99 1.93	2.76 2.75
Cottonseed meal III	1.90 2.04	2.76 2.82
Cottonseed meal IV	2.02 2.10	2.83 2.78
Cottonseed meal V	2.12 2.07	2.86 2.71
Cottonseed meal VI	1.96 2.00	2.97 2.89

TABLE V

RATIONS USED IN THIRD COTTONSEED MEAL-HULL BRAN STUDY

Marcol B-75 oil	2.0	2.0	2.0	2.0	2.0	2.0
Yellow corn meal	58.5	61.3	60.0	58.5	56.7	54.7
Soybean meal (45% protein)	25.0					
CSM (50% protein)		22.2				
CSM (48.8% protein)			23.5			
CSM (47.1% protein)				25.0		
CSM (46.3% protein)					26.8	
CSM (44.9% protein)						28.8
Fish meal	3.0	3.0	3.0	3.0	3.0	3.0
Alfalfa leaf meal	3.0	3.0	3.0	3.0	3.0	3.0
Steamed bone meal	2.0	2.0	2.0	2.0	2.0	2.0
Oyster shell flour	1.0	1.0	1.0	1.0	1.0	1.0
Salt	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix	2.0	2.0	2.0	2.0	2.0	2.0
Dried Whey	3.0	3.0	3.0	3.0	3.0	3.0

Vitamin premix supplies per ton:

4 grams riboflavin, 8 grams pantothenic acid, 18 grams niacin, 5,300,000 Units vitamin A, 30,000 AOAC chick units vitamin D_3, 4 grams procaine penicillin, 6.25 milligrams vitamin B_{12}, 0.50 pounds DL-methionine.

TABLE VI

SUMMARY OF RESULTS OF THIRD COTTONSEED MEAL-HULL BRAN EXPERIMENT

Ration	Average Weight	Feed Efficiency
	8 weeks	8 weeks
Soybean Meal (46% Protein)	2.03	2.59
	2.03	2.54
Cottonseed Meal (50% Protein)	1.84	2.58
	1.94	2.55
Cottonseed Meal (48% Protein)	1.85	2.54
	1.98	2.59
Cottonseed Meal (47.1% Protein)	1.94	2.52
	1.94	2.66
Cottonseed Meal (46.3% Protein)	1.96	2.57
	1.86	2.62
Cottonseed Meal (44.9% Protein)	1.86	2.73
	1.90	2.65

Discussion

Altschul: What about effect of crude fiber in the ration? Aines says cottonseed meals and soybean meal cannot be compared due to differences in fiber content. Would the 50:50 mixtures in the rations have been equal to the control ration if all rations were fed at the same fiber content level?

Watts, Waddell and Stephenson: We disagree with your statement.

Cavanaugh: We prepared cottonseed meals of 49-50% protein content by screening techniques. We could increase protein content of meals and concurrently decrease fiber content.

Frampton: Regression coefficients for relation of crude fiber to weight gains were 0.8, 0.86 and 0.78. At odds of 100 to 1, the coefficient is 0.6.

Watts: We conducted tests with rice hulls. When rations were isocaloric, fiber seemed to produce no effect. Perhaps fiber from cottonseed hulls would not be effective if fed in isocaloric rations.

Grau: Dr. Frampton, do your regression coefficients refer to rations containing all 3 levels of cottonseed meals?

Frampton: Yes.

Altschul: Two more points for discussion: (1) supplementation of diets containing cottonseed meal with lysine brought weight gains up to that observed with control soybean meal diet; (2) when cottonseed meal rations had deteriorated during storage, addition of lysine raised their nutritive value but did not increase value to control level.

Stokes: Addition of lysine to cottonseed rations did not bring them to level of control in our studies.

Eagle: Heidebrecht has stated that female chicks were heavier after 8 weeks feeding trial than male chicks. Perhaps one would have to consider sex of birds to determine lysine requirement.

Ryan: From viewpoint of feed manufacturer, the suggestion of leaving out hulls from cottonseed meals is most sensible. The manufacturer does not want to pay 8 cents a pound for fat to equalize hull fiber content in the meals.

Heidebrecht: Was a different source of soybean meal used in each trial in studies of Dr. Watts?

Watts: Yes.

Curtin: Was source of hulls same in No. 2 and No. 3 experiments?

Watts: No. Hull bran for No. 2 study was supplied by Western Cottonoil Company, that for No. 3 was a blend of 2 hull samples. There is some evidence that grinding of hulls lowers effectiveness of fiber.

Ryan: In your second experiment, Table IV, only soybean meal gave good growth for chicks. In Table VI of your data, all rations, including the soybean meal control, supported poor growth. I don't think you have a critical test for effect of fiber in rations under such conditions of poor growth.

Watts: Faster rate of growth than 2.0 to 2.5 pound growth in 6 weeks would give a better test for effect of fiber in the diet. Even an 80:20 ration allowing for more growth would be more critical test.

Barnett: From data it appears that there is no relationship between feed consumption and fiber content. At 50:50 levels, the cottonseed meal:soybean meal rations supported as much growth as the control soybean meal ration. Fiber content did not exert any effect.

Watts: How can you separate fiber effects from those of protein contents since fiber content changed concurrently with changes in protein content?

Barnett: Fiber may be important, but we have not shown it from this cooperative study.

Rusoff: Suggest you use terminology of "lignin" or "cellulose" constituents of cottonseed hulls, instead of crude fiber when you are talking about chick rations.

Watts: Cottonseed hulls contain high lignin content; but trade still uses expression "crude fiber."

Stephenson: You must remember that chicks are poor users of cellulose, whereas ruminants utilize cellulose.

Seminar II - PROTEIN QUALITY AND GOSSYPOL TOXICITY

Chairman - H. E. Robinson, Swift and Company, Chicago, Illinois

TOXICITY OF FREE GOSSYPOL IN RELATION TO PROTEINS OF FEED

F. H. Smith
North Carolina Agricultural Experiment Station
Raleigh, North Carolina

The varied growth response of pigs and of rats fed different cottonseed meals having the same level of free gossypol indicated that the toxicity of gossypol is not clearly defined and the quality of proteins is involved.

Re-investigation of the toxic levels of gossypol for pigs included a series of collateral experiments with weanling rats:

Experiment 1

The effect of different levels of free gossypol on the growth response of weanling rats was evaluated for diets containing 17.4% crude protein, supplied by either soybean meal or casein. The two basal diets contained, respectively: (1) corn, 70.3%; soybean meal, 21.0%; and alfalfa meal, 5.0% and minerals and vitamins, 3.7%; (2) casein, 17.4%; fat, 5.0%, including 0.5% cod liver oil; Wesson salt, 4.0%; and starch, including vitamins, 73.6%. Other diets were prepared to contain 0.075% and 0.150% free gossypol by replacing soybean meal (1) or casein (2) with petroleum ether-extracted cottonseed meats. A third series of diets, containing the foregoing levels of free gossypol, were prepared by direct addition of pure gossypol to the casein basal diets (2).

Summary: There were no significant differences between the responses to the basal diets. Gossypol at a given level had essentially the same effect in all diets, but responses from different levels were highly significant.

Experiment 2

The effects of different proteins on the toxicity of free gossypol were evaluated by the growth of weanling rats over a 4-week period. The proteins, constituting 16% of the diet, were (1) solvent-extracted soybean meal, (2) ether-extracted cottonseed meats (low gossypol), (3) commercial cottonseed meal (low gossypol), and (4) same as (3) plus 1% L-lysine. Diets were prepared from each of the respective proteins to contain 0.00%, 0.075% and 0.150% free gossypol.

No added gossypol: Rats fed either the ether-extracted cottonseed meats (2) or the cottonseed meal plus lysine (4) made the greatest and about equal weight gains, followed in order by soybean meal (1) and cottonseed meal (3).

Gossypol at 0.075%: The observed gains from the diets in descending order were: soybean meal (1), cottonseed meats (2), cottonseed meal plus lysine (4) and cottonseed meal (3). The mean differences were marked except between the last two diets listed.

Gossypol at 0.150%: The mortality of rats fed the commercial cottonseed meal diets (3,4) was 100% within 2 weeks. Of the remaining two diets, only one death occurred, which was in the soybean-fed (1) group. Though all rats of the ether-extracted cottonseed meats group (2) survived, their mean weight gain was markedly lower than that from soybean meal (1).

These data indicate that the apparent toxicity of gossypol, as measured by mortality and by gains in body weight of the young rat, is affected by the quality of the protein used in the test diets. The performance of weanling rats fed free gossypol in diets containing different proteins at the same level provides a biological method for evaluating the quality of the different proteins.

Experiment 3

Cottonseed meals Nos. 10, 16, 19, 45 and 49, supplied by the Southern Regional Research Laboratory, were fed at a 16% protein level to weanling rats for 28 days. Meals Nos. 10, 45 and 49 were low in free gossypol, No. 16 was intermediate, and No. 19 was high. Ether-extracted cottonseed meats were used to supply the protein in the control diet. Sufficient refined cottonseed oil (Wesson) was added to provide a fat content of 10%. Adequate minerals, vitamins and starch were incorporated to complete the diet.

Similar diets containing 0.075% free gossypol were prepared from each of these meals except No. 19, which exceeded this value when fed at the 16% protein level to evaluate the protein quality when fed under the stress of the increased level of gossypol. The results are presented in Table I.

Without added gossypol: It will be noted from Table I that the weight gains from cottonseed meal No. 49 were greater and those from meal No. 45 were somewhat less than the gains from the control. Meals Nos. 10, 16 and 19 produced about the same weight gains, which were markedly lower than that of either the control or the other two meals.

With 0.075% free gossypol: The additional gossypol had little effect on the response from meal No. 49 and somewhat more on the control. The latter, with added gossypol, gave growth superior to meals Nos. 10, 16 and 19 without the added gossypol. The added free gossypol adversely affected the growth response from meals Nos. 10, 16 and 45 by 37%, 38% and 51% respectively.

There were no differences between meals Nos. 10 and 16 as measured by the growth response of rats. The effects of 0.075% free gossypol in the diets from the other meals indicate that meal No. 19 is toxic at the level fed. Meal No. 45 was different from the control and meal No. 49. The latter was only slightly affected by the added gossypol. These data indicate that factors other than free gossypol in cottonseed meal, presumably protein quality, affected the growth response of the weanling rats to a marked degree. The highest nitrogen solubility was observed in meal No. 49 and the ether-extracted cottonseed meats which gave the greatest weight gains. Prepressed solvent extracted meal No. 45, with the higher nitrogen solubility and lysine content, was superior to prepressed solvent extracted meal No. 10.

Experiment 4 - The Effect of Bound Gossypol in Dietary Protein.

Pure gossypol was bound to solvent extracted soybean meal, ether-extracted cottonseed meats and solvent extracted peanut meal to the extent of 1.01, 1.10 and 1.06 percent respectively. The combination was carried out under mild conditions in order not to affect the protein quality.

The diets were fed to weanling rats for 28 days at the 10% protein and 5% fat levels. Starch was added to complete the diets after adequately supplementing them with minerals and vitamins.

The soybean meal and cottonseed diets were individually fed to 10 rats according to a randomized block design. The peanut meal diets were fed to 5 rats according to the same plan.

The results of this study are presented in Table II. This study shows that the growth response of weanling rats is lowered to a highly significant degree when gossypol is bound to soybean and cottonseed protein to the extent of about 1% and fed at the 10% protein level. The effect of gossypol bound to peanut protein was significant; however, the growth response from all the peanut meal diets were markedly lower than that from either the soybean meal or cottonseed meats diets. The bound gossypol did not reduce the gains from the soybean meal diet to the same degree that those from the cottonseed meats diet was lowered.

The data indicate that the binding treatment had no significant effect on the protein quality of any of the meals.

This study definitely shows that bound gossypol affects the quality of protein.

TABLE I

WEIGHT GAINS OF WEANLING RATS FED COTTONSEED MEALS, PRODUCED BY DIFFERENT PROCESSING METHODS, AT 16% PROTEIN LEVEL FOR 28 DAYS WITHOUT AND WITH 0.075% FREE GOSSYPOL ADDED

Cottonseed Meal No.	Processing Method	Temp. °F.	Nitrogen Solubility	Lysine	Gossypol		Gains Added Gossypol	
					Free	Total	0%	0.075%
			%	%	%	%	gm.	gm.
Cottonseed Meats	Ether-extr.	---	95.1	--	0.05	0.34	108.7	78.7
10	P.P.S.E.	245	52.40	3.42	0.04	1.00	60.8	38.0
16	Hydr.	234	65.09	3.64	0.14	1.11	65.1	40.1
19	Solv. E.	240	70.00	3.87	0.28	0.96	58.1	--
45	P.P.S.E.	---	65.90	3.86	0.04	0.75	95.3	46.5
49	Degossypolized	---	83.66	--	0.04	0.84	113.3	100.7

TABLE II

EFFECT OF GOSSYPOL BOUND TO DIETARY PROTEIN ON WEIGHT GAINS OF WEANLING RATS (16% PROTEIN LEVEL, 28 DAYS)

Source of Protein	Treatment of Protein		
	None	For Binding	
		No Added Gossypol	Added Gossypol
	gms.	gms.	gms.
Soybean Meal 1/	58.1	60.3	45.2
Cottonseed Meats 1,2/	63.1	57.3	37.0
Peanut Meal	23.7	23.4	6.8

1/ L.S.D. .05 9.49; L.S.D. .01 13.80

2/ Diethyl-ether extracted meats prior to feeding study.

EFFECT OF PROTEIN SOURCE AND GOSSYPOL LEVEL IN THE RATION ON GROWING PIGS

A. J. Clawson and E. R. Barrick
North Carolina State College
Raleigh, North Carolina

The toxicity of gossypol in cottonseed meal for swine has been known for over 25 years (Hale, 1930; Robinson, 1934). Poultry and swine feeding experiments with cottonseed meals processed to be of low free gossypol content have given somewhat variable results, presumably due to differences in quality of the protein in the meals. The gossypol content of cottonseed meal used in much of the early research was not reported. If cottonseed meals are to be used extensively in swine rations, it is essential to have reliable information concerning their free gossypol content and the gossypol tolerance level of swine. Information on the effects of protein quality or source of protein on free gossypol tolerance is lacking.

The purposes of this study were to more accurately define the level of free gossypol that causes growth depression and toxicity in young growing pigs and to evaluate the effect of protein source on gossypol tolerance.

In the first experiment a corn-soybean meal ration to which graded levels of free gossypol were added was used. The ration used contained 70.3% ground corn, 21% soybean meal, 5% dehydrated alfalfa meal, 2% deflourinated phosphate, 0.7% limestone, 0.5% vitamin-antibiotic premix and 0.5% trace mineral salt. The indicated free gossypol levels were obtained by addition of hexane extracted cottonseed meats. The prepared meats contained 0.93% of free gossypol and were added to the diets to replace an equal weight of soybean meal. The free gossypol content of the complete rations was checked by chemical analysis.

The results of the first experiment comparing the effect of graded levels of free gossypol in corn-soybean meal rations are presented in Table I. No symptoms of gossypol toxicity were observed and growth rate was apparently not affected by free gossypol at the levels fed.

TABLE I

THE EFFECT OF GOSSYPOL LEVEL ON GROWTH OF PIGS*

Gossypol level in ration, %	Av. daily gain, lbs.	Feed per cwt. gain, lbs.
.000	1.54	294
.003	1.51	268
.006	1.50	299
.009	1.49	273
.012	1.40	318
.015	1.49	290
.018	1.53	297
.021	1.61	342

* Initial wt. 33 lbs.; final wt. 119 lbs.; 3 pigs per treatment.

Failure of the gossypol fed in this test to cause symptoms of gossypol toxicity prompted the use of even higher levels. Table II shows the results of a second test with doubled gossypol levels.

TABLE II

EFFECT OF GOSSYPOL LEVEL IN THE RATION ON GROWTH OF PIGS*

Gossypol level in ration, %	Av. daily gain, lbs.	Feed per cwt. gain, lbs.
.000	1.57	214
.006	1.44	258
.012	1.53	254
.018	1.52	211
.024	1.48	229
.030	1.43	200
.036	1.35	279
.042	1.46	255

* Initial wt. 26 lbs.; final wt. 132 lbs. 2 pigs per treatment.

No symptoms of gossypol toxicity were observed even at the higher levels of gossypol. Gossypol toxicity normally occurs in pigs fed corn-cottonseed meal rations at much lower levels of free gossypol than were used in this test. This would indicate that either protein quality has an influence on gossypol toxicity or that soybean meal contains a factor other than protein which in some way increases the pig's tolerance to gossypol.

Experiment 2 was conducted to compare the effect of source of protein on gossypol tolerance. Two sources of cottonseed meal were used. No. 1 was a pre-press solvent extracted meal containing 0.04% free gossypol and No. 2 was an expeller meal containing 0.09% free gossypol. Each cottonseed meal contained 41% protein and the soybean meal contained 47% protein. Cottonseed meats, as obtained from the flaking rolls and containing 0.99% free gossypol was added to the rations to obtain the desired levels of free gossypol. Table III shows the results of this comparison.

TABLE III

EFFECT OF PROTEIN SOURCE AND GOSSYPOL LEVEL IN THE RATION ON GROWING PIGS*

Experiment 2, Fall, 1955 (85 days on test)

Protein Source	No. pigs	Gossypol level, %	Av. daily gain, lbs.	Feed/cwt. gain, lbs.	Toxicity symptoms
S.B.M.	8	0.000	1.63	366	none
S.B.M.	8	0.008	1.64	374	none
S.B.M.	8	0.013	1.60	385	none
S.B.M.	8	0.018	1.57	376	none
S.B.M.	3	0.036	1.21	452	none
S.B.M.	3	0.071	0.74	397	100%
C.S.M. No. 1	8	0.008	1.09	438	37%
C.S.M. No. 2	8	0.018	1.02	465	100%
C.S.M. No. 1 and 2	8	0.013	1.21	421	25%
C.S.M. No. 1 and S.B.M.**	3	0.013	1.70	379	none
C.S.M. No. 1 and S.B.M.**	8	0.018	1.50	373	none
C.S.M. No. 2 and S.B.M.	3	0.004	1.57	398	none

* Average initial and final wts. 35 and 152 respectively.
** Cottonseed meals added to adjust gossypol level.

Mixtures of half cottonseed and half soybean meal prcduced gains nearly equal to soybean meal alone at all levels of gossypol fed. In this experiment higher levels of gossypol were tolerated without causing toxicity in corn-soybean meal rations than in corn-cottonseed meal rations.

Experiment 3 was conducted to compare the effects of processing procedures used in preparation of cottonseed meals on growth rate and gossypol tolerance of young pigs. Meals furnished by the National Cottonseed Products Association were used. The growth performance of pigs fed the different meals in complete rations containing 16% protein was quite variable (Table IV) and did not seem to be closely related to the free gossypol content of the rations fed. This indicates that factors other than free gossypol are probably involved.

TABLE IV

EFFECT OF PROCESSING CONDITIONS AND FREE GOSSYPOL LEVEL ON GROWTH OF PIGS*

Meal identification	Processing procedure	Gossypol level in ration, %	Av. daily gain, lbs.
C.S.M. 19	Solvent extracted	0.045	0.49**
C.S.M. 10	Prepress solvent	0.007	1.18
C.S.M. 16	Hydraulic	0.022	1.18
C.S.M. 45	Prepress solvent	0.007	1.46
C.S.M. 49	Solvent degossypolized	0.003	1.71
S.B.M.	Solvent	--	1.75

* Three pigs per treatment, av. initial wt. 33 lbs.; av. final wt. 185 lbs.

** 100% mortality

Summary

1. From the data it appears that higher levels of free gossypol can be tolerated by pigs in corn-soybean meal rations than in corn-cottonseed meal rations.

2. Rations containing equal parts of soybean and cottonseed meals as a source of protein supplement produced growth in pigs equal to soybean meal alone when fed at the same protein level.

3. Growth performance of pigs fed different cottonseed meals was quite variable and was not closely related to the free gossypol content of the rations fed.

GOSSYPOL TOXICITY AND PROTEIN LEVEL IN THE NUTRITION OF THE CHICK AND LAYING HEN

Ram Narain, C. M. Lyman and J. R. Couch
Departments of Poultry Science and Biochemistry and Nutrition,
Texas A. and M. College System
College Station, Texas

Chick Studies

A synthetic type diet composed of soybean protein and glucose monohydrate and supplemented with vitamins and minerals at levels thought to be adequate was used in two experiments with New Hampshire chicks in order to determine the effect of protein level on free gossypol toxicity. The 0.04% level of free gossypol appeared to be toxic with protein levels of 17 and 21% and was increased to 0.06% with the 42% protein level. The growth of the chicks used in these studies was increased as the level of protein was increased from 17 to 42% at four weeks of age. The statistical analysis of the weights of the birds revealed a significant interaction between protein level and gossypol level.

It was further observed that the hemoglobin content of the blood decreased as the gossypol level of the diet increased in groups fed 17 and 21% protein. Such an effect on the hemoglobin content of the blood was not observed when the diet contained 42% protein.

Paper electrophoretic studies were performed with a Spinco Model R paper electrophoresis apparatus, using the serum of six week old normal chicks and chicks fed 0.1% free gossypol. The procedure used was that recommended for serum by the manufacturer of the apparatus employing a barbiturate buffer at pH 8.3, ionic strength 0.075 with a running time of 16 hours at a current of 5 milliamperes. Quantitative estimation of the percentages of each fraction present was accomplished by scanning with a Spinco Analytrol and calculation of the area under the curve of each fraction. Paper electrophoretic patterns from normal chicks showed sharp, tall albumin peaks and a higher serum total protein content as measured by total area under the curve than did the chicks fed 0.10% free gossypol.

Albumin and globulin were determined chemically on serum from four week old New Hampshire chicks in the second study. There was a tendency toward an increase in serum albumin and globulin as the protein content of the diet was increased.

Hen Studies

Two experiments were conducted with six White Leghorn hens per group. The birds were maintained in individual cages with raised screen floors. Levels of 17.5 and 35% protein were used. Soybean meal was used as the protein in the first study and fish meal was the protein concentrate in the second study. The average weights of the hens were decreased as the level of gossypol in the diet was increased in both studies. Egg production was severely affected and ceased entirely in the high gossypol fed groups. The egg weight was also reduced as the gossypol level in the diet was increased. The gossypol-cephalin content of the egg was reduced as the protein content of the diet was increased in the experiment where fish meal was used as the protein source, but was not so apparent where soybean meal was used as the protein source.

The feed consumption of hens fed the higher levels of gossypol was decidedly reduced. This, no doubt, accounts in part for the loss of weight and other adverse effects observed by feeding the higher levels of gossypol.

TOXICITY AND PROTEIN QUALITY STUDIES ON COTTONSEED

E. Eagle
Swift and Company
Chicago, Illinois

The effect of prolonged storage of cottonseed pigment glands and pure gossypol on the acute oral toxicity in the rat and on the gossypol content was determined. The effects of incorporation of pure gossypol or cottonseed pigment glands at a constant gossypol level were determined in a rat feeding test. The relationship between biologically evaluated toxicity and protein quality of cottonseed meals in the rat and the gossypol content and percent nitrogen solubility will be discussed.

RELATION OF QUANTITY AND QUALITY OF DIETARY PROTEIN TO TOLERANCE OF THE GROWING PIG FOR FREE GOSSYPOL

I. P. Earle and J. W. Stevenson
Animal and Poultry Husbandry Research Branch
Beltsville, Maryland

Past studies designed to determine the maximum tolerance of the growing pig for free gossypol, have shown a marked lack of agreement between results obtained in different experiments. The discrepancies have appeared to be related to some variable, other than free gossypol, in the specific cottonseed meals used in the diets. For example, in

one series of experimental diets, levels of free gossypol as high as 0.024% have been well tolerated; in another series which utilized a different meal, typical gossypol toxicity has occurred at a level as low as 0.011%.

The present experiment was undertaken to verify the hypothesis that there is a relation between gossypol tolerance and dietary protein operating in the growing pig which is similar to that which has been demonstrated in the protein-depleted rat. To this end, observations have been made on differences in the rate of growth and in the incidence of toxicity in groups of weanling pigs fed 9 different diets which have utilized 3 cottonseed meals, differing in protein quality as assayed by both a rat growth method and a rat protein-repletion method. Each meal has been fed at 3 different levels, the same level of free gossypol (0.015%) being maintained in all diets. The results have demonstrated a marked beneficial effect of both increased protein level and improved protein quality on resistance to gossypol toxicity. Differences in rate of growth of the pigs before toxicity intervened corresponded well with the differences between meals, indicated by assay of protein quality by the rat growth procedure. Differences in resistance to gossypol toxicity of the pigs fed the 3 meals appear to be more nearly related to protein values of the 3 meals when assayed by means of the rat repletion method and suggest that those characteristics of cottonseed meal protein which appear to influence gossypol tolerance may not be identical with those characteristics which influence the value of the protein for promotion of growth.

EFFECT OF VARYING PROTEIN INTAKE, SOURCE AND LYSINE CONTENT ON FREE GOSSYPOL TOLERANCE BY THE RAT

C. A. Cabell
Animal Husbandry Research Division
Agricultural Research Service
Beltsville, Maryland

Previous experiments reported from this laboratory have indicated that free gossypol tolerance by the protein depleted rat is related to both level and quality of protein.

The object of this report is to review certain relationships between crude protein intake, protein source and gossypol tolerance by protein depleted rats and to show some data indicating that lysine content may be involved in this relationship.

Briefly, the work has been done with groups of mature male rats. The animals are first depleted of protein by feeding a low protein diet, then repleted by feeding the basal diet supplemented with an exact daily intake of protein and an estimated intake of gossypol.

It has been found by this technique that with some protein supplements fed at a daily protein intake of 1 gram, that a simultaneous intake of 9.5 mg. of free gossypol produces a significant depression in mean weight gain in the rats.

When this 1 gram of protein is of good biological value, the animals will average about 15 grams daily food consumption.

Figure 1 shows the effect of varying the protein intake at this constant level of 9.5 mg. of free gossypol. The source of gossypol in this feeding trial and others which have given similar results was a high gossypol cottonseed meal and there is an indication here that gossypol tolerance is affected by protein intake. At the low protein intake of 1 gram per day, a significant weight depression is evident. However, this does not occur when the protein is increased to 1.5 or 2.0 grams.

The critical levels of 9.5 mg. of gossypol and 1 gram of protein might be expected to vary some with protein source or quality. The data illustrated in Figure 2 indicate that such is the case. All of these proteins were fed at the same daily intake of 1 gram of crude protein (N x 6.25), both with and without a daily intake of 9.5 mg. of free gossypol. Here again the source of gossypol was an experimental high gossypol meal.

These data show that gossypol fed with one of the cottonseed meals and with soybean meal failed to produce a significant depression in weight gain. With casein and two of the cottonseed meals the difference is found to be statistically significant. Calculated 5% level least significant mean differences in these feeding trials usually fall between 2.5 and 3.5 grams.

These results are important in their relation to swine feeding experiments. The two protein sources that gave weight depression in rats produced toxic symptoms when fed to swine at relatively low levels of free gossypol. However, the same estimated protein and free gossypol intake was well tolerated by swine when the protein source was the soybean meal or the HV cottonseed meal. The results indicate that weight gain of the rats and toxicity in swine are being affected by the same factors.

Although it has been possible to increase weight gains of the rats by supplementary amino acids, no combination was found which would eliminate weight depression due to this critical level of free gossypol. Low levels of dl-lysine Hydrochloride were especially ineffective.

A series of cottonseed meals provided by the Southern Regional Research Laboratory offered an opportunity to test the effect of naturally-occuring lysine on gossypol tolerance. Lysine content of these meals was determined at that laboratory by an ion exchange procedure and these values were made available with the meal samples. Figure 3 shows results obtained with the rat repletion method using a daily protein intake of 1 gram, both with no added gossypol and with an intake of 9.5 mg. per day added in the form of pigment gland meal.

The data show an apparent inverse relationship between lysine content of meals and weight depression due to free gossypol. Weight depression is only 20% at the high lysine level but is more than 80% when the low lysine meal is fed. Further, there is some indication that depression of weight gain is more highly negatively correlated with lysine content than with biological value. While meal No. 440 has lowest lysine and highest weight depression from free gossypol, it does not have lowest biological value when no gossypol is added.

PROTEIN VS. GOSSYPOL TOLERANCE

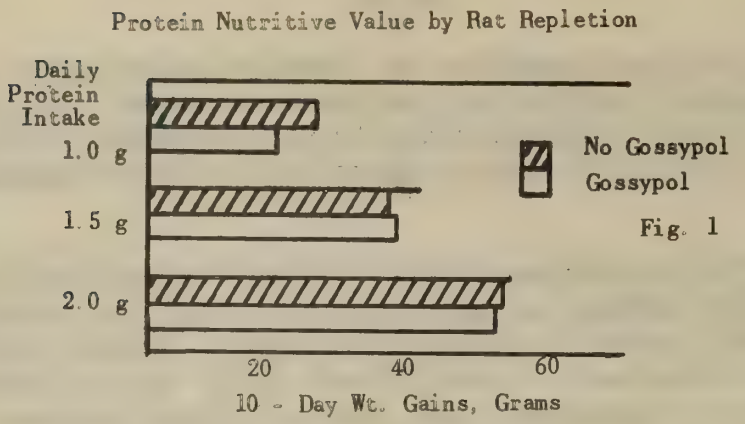


Fig. 1

Effect of increasing protein intake on free gossypol tolerance by protein depleted rats

PROTEIN SOURCE VS. GOSSYPOL TOLERANCE

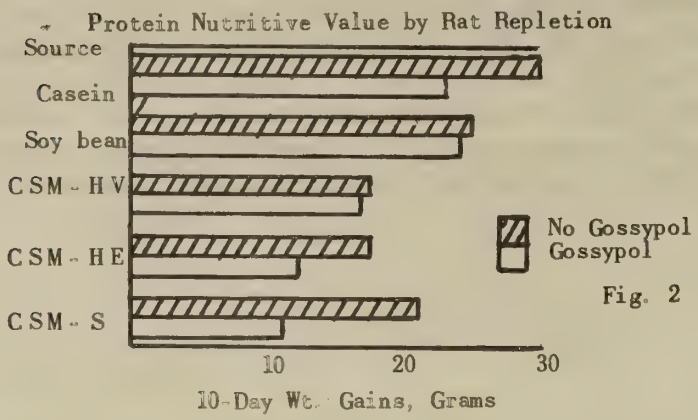


Fig. 2

Variation in free gossypol tolerance with protein from different sources

LYSINE VS. GOSSYPOL TOLERANCE

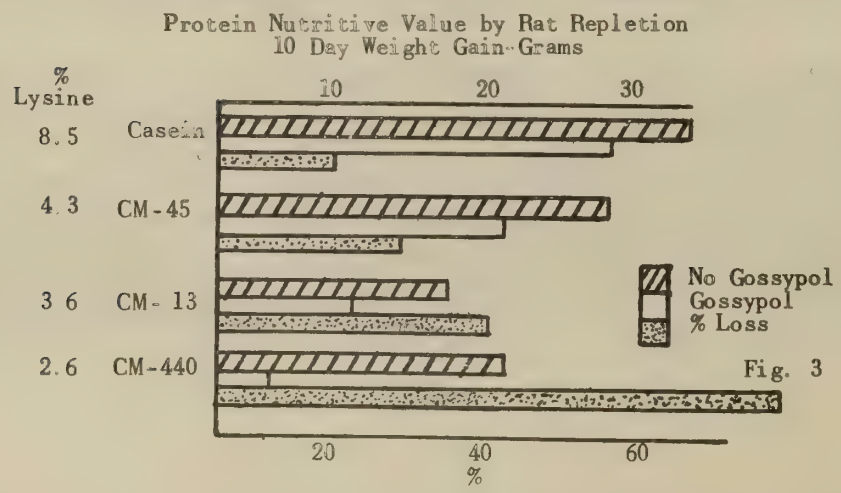


Fig. 3

Effect of lysine content of meal on free gossypol tolerance by protein depleted rats

PROTEIN QUALITY EVALUATIONS OF CODED COTTONSEED MEALS IN THE RAT AND CHICK

E. Eagle
Swift and Company
Chicago, Illinois

Evaluations of the protein quality of a series of nine coded cottonseed meals were made in two rat tests and two chick tests, with soybean meal and butanone-extracted cottonseed meal as the controls in the former, and soybean meal as the control in the latter. Efficiencies of protein utilization, feed utilization and caloric utilization were determined in each of the four tests after both four weeks and eight weeks. There is poor correlation between the nitrogen solubility values and the protein quality of the nine meals as determined biologically in the rat at the 9% protein level and in the chick at the 15% level. The data obtained on these nine samples are in agreement with our previous findings on a series of twenty-five samples (Eagle, et al., J. Am. Oil Chemists' Soc., 1956, 33:15-21).

Discussion

Heywang: Are the pathological spleen data from rat specimens?

Eagle: Yes.

Altschul: I think at this point that we are justified in maintaining that cottonseed meals of 75% or more nitrogen solubility are good for incorporation into chick rations.

Eagle: I have data which show that meals of 70% nitrogen solubility support as much growth as those of 75% nitrogen solubility. We may have to lower our standards on requirements of nitrogen solubility in cottonseed meals.

Rusoff: Does Eagle have nitrogen solubility data to tie in with the lysine data?

Eagle: I do not know what the total lysine content of cottonseed meal implies in regard to nutritive value. But I do wonder about the relation of nitrogen solubility and what it means. From 104 analyses performed in different laboratories the values for nitrogen solubility content of one meal ranged from 44.8 to 63.5 percent; the grand average of these values was 56.9

percent. With another sample of meal subjected to 104 analyses at different laboratories, a range of 61.8 to 79.6 percent nitrogen solubility was obtained, and the grand average in that case was 74.0 percent nitrogen solubility. I wonder what analyses for nitrogen solubility really means.

Eagle: Do we have sufficient evidence to state that bound gossypol is not toxic?

Lyman: We know that when free gossypol is removed from cottonseed meals, the meals are not toxic.

Eagle: Well, I fed a sample of gossypol-protein complex which contained a 1:1 ratio of gossypol to protein. That substance was toxic. How do you explain this if bound gossypol is not toxic?

Altschul: At the time we supplied the sample of gossypol-peanut protein complex to you, we were interested in finding a product whose toxicity would approach that of gossypol in the cottonseed pigment glands. One of the reasons we believe gossypol in the pigment glands is more toxic than isolated, purified gossypol may be due to the fact that gossypol in the pigment glands exists as a water-dispersible pigment. By processes of isolation and purification, gossypol is rendered water-insoluble. Perhaps in the case of the 1:1 ratio of gossypol to peanut protein in the complex, there were not sufficient protein groups available for binding and detoxifying the gossypol. You may recall that feeding of gossypol-protein complexes which contained smaller amounts of gossypol and more of protein produced no toxic effects even though the gossypol content, if in "free" state, would have been toxic. In the case of gossypol-amino acid combination products of the 1:1 ratio of gossypol: amino acid, there are more groups immediately available for combining with the gossypol. You may recall from the work of Kuiken, Heywang and Couch that a combination product of 1:1 ratio of gossypol:lysine was not toxic.

Eagle: I fed a 1:9 gossypol-glycine combination product to rats, and it was not toxic. Then I injected the same product intraperitoneally to dogs. I was interested in determining the effects, if any, of such a compound on heart rate, blood pressure, EKG, and respiration. Before the kymograph recordings had proceeded more than 14 minutes,

the dog died. I was wondering whether intraperitoneally injected gossypol-glycine was really toxic to the dog and not toxic to the rat. When I injected pure glycine into dogs, they likewise died. It was evident that glycine was the toxic factor and the combined gossypol-glycine was less toxic peritoneally than pure glycine. This should be a reminder to all of us that over-supplementation of cottonseed meals with pure amino acids may increase the toxic symptoms of the meals. I think the toxicity of gossypol-glycine to goldfish may be explained on the basis of the toxicity of the glycine contained therein.

Berardi: Yes, all of the water-soluble gossypol-protein and gossypol-amino acid complexes killed the fish by clogging their gills. The fish grew very well when given a diet of peanut protein. However, we did feed gossypol-glycine to rats, mice and rabbits. It was not toxic. We have published a paper describing the non-toxicity of gossypol-glycine to mice. The reference is: Arch. Biochem. Biophys., 44, 181-188 (1953).

Seminar III - CHEMICAL MEASURES OF NUTRITIVE VALUE

Chairman - C. M. Lyman, Texas A&M College, College Station, Texas

LYSINE CONTENT OF COTTONSEED MEALS

W. H. Martinez and V. L. Frampton
Southern Regional Research Laboratory
New Orleans, Louisiana

By means of the Moore and Stein ion exchange procedure for amino acids, the lysine content of the series of cottonseed meals used in the nutritional experiment was determined. A variation in the lysine content of commercially produced cottonseed meals was observed. There was a variation in lysine content from a high of 4.2 grams of lysine per 16 grams of meal nitrogen for an unheated meal prepared in the laboratory from prime seed to a low of 2.3 grams for an excessively heat damaged commercial meal. Most of the values for lysine of commercially prepared cottonseed meals used in the collaborative nutrition experiment ranged between 3 and 4 grams per 16 grams of nitrogen. See Table I for results.

Processing obviously affects the lysine level of the meals, but the variations in operating any given process are so great that simply naming the process is not sufficient for characterizing the meal.

In the same manner the lysine content of the diets used in the nutrition experiment was determined. A definite correlation between lysine content of the diets and the growth response of the chickens was noted. These data are tabulated in Table II.

TABLE I

LYSINE CONTENT OF ACID-HYDROLYZED COTTONSEED MEALS

Cottonseed meal number	Lysine, g. per 16 g. of nitrogen	Process
S6,76	4.3	Experimental meal
CM-6	4.0	Prepress solvent-extracted
CM-21	3.9	Low speed screw-press
CM-45	3.9	Prepress solvent-extracted
CM-19	3.9	Solvent-extracted
CM-36	3.6	Low speed screw-press
CM-16	3.6	Hydraulic-press
CM-10	3.4	Prepress solvent-extracted
CM-13	3.4	High speed screw-press
CM-440	2.3	Excessively heat damanged, screw press

TABLE II

LYSINE CONTENT OF DIETS AND WEIGHT OF BIRDS AT 8 WKS.

Meal	Series 1[1] Lysine g/g. of diet x 10^{-2}	Series 1[1] Weight[4] g.	Series 2[2] Lysine g/g. of diet x 10^{-2}	Series 2[2] Weight g.	Series 3[3] Lysine g/g. of diet x 10^{-2}	Series 3[3] Weight g.
13	0.701	330	0.774	727	0.955	1150
16	0.709	505	0.814	850	0.904	980
36	0.737	547	0.867	937	0.983	1127
19	0.761	600	0.936	846	---	963
21	0.779	781	0.829	1085	0.959	1228
45	---	849	0.925	1068	---	1171
50[5]	1.025	1287				

1/ Diet contains 100% cottonseed meal.
2/ Diet contains 75% cottonseed meal - 25% soybean meal.
3/ Diet contains 50% cottonseed meal - 50% soybean meal.
4/ Average weight of Cockerels from all stations at 8 weeks.
5/ Standard Diet 50 contains 100% soybean meal.

ε-DNP LYSINE DETERMINATIONS IN COTTONSEED MEALS

E. J. Conkerton
National Cottonseed Products Association Fellow
Southern Regional Research Laboratory
and
V. L. Frampton
Southern Regional Research Laboratory
New Orleans, Louisiana

As data were collected during the Nutrition Experiment, it became apparent that the lysine content of the meal was an important factor in its nutritive value. Therefore, experiments were set up to devise a more rapid chemical method for the determination of lysine, than the ion-exchange chromatographic technique now used. The method which had been selected involves reaction of a bicarbonate suspension of the whole meal with an alcoholic solution of 2,4-dinitrofluorobenzene, overnight acid hydrolysis of the resulting dinitrophenyl protein, separation of the water and ether soluble dinitrophenyl amino acids, and spectrophotometric determination of the ε-dinitrophenyl lysine content of the water soluble fraction. From this value, the free ε-amino lysine content of the meals can be calculated. In several of the meals analyzed, the total and free ε-amino lysine contents were similar (Table I); however, in a few the free ε-amino lysine was lower than the total lysine content. For example, CM-10 and CM-13 each have a 3.4% lysine in the protein but their free ε-amino lysine contents are 3.3 and 2.3%, respectively. CM-13 was poorer nutritionally than CM-10. the DNFB method offers a relatively rapid technique for the determination of lysine in cottonseed meals and may, in addition, be useful in differentiating between two meals of similar total lysine content but of varying nutritional quality.

TABLE I

LYSINE CONTENTS OF SELECTED COTTONSEED MEALS

Description of Meal	Percent Lysine in Protein	
	Chromatographic method	DNFB method
Hexane-extracted	4.2	4.4
Butanone-extracted	4.3	4.3
CM-19*	3.9	3.9
CM-6*	4.0	3.6
CM-10*	3.4	3.3
CM-13*	3.4	2.3

* Meals used in Nutrition Experiment.

Discussion

Lyman: We used the same procedure for lysine determination as Mrs. Conkerton did. I do think that the method of analysis could stand improvement.

Waddell: What was the hydrolysis period?

Conkerton: Samples were hydrolyzed for 16 hours. That period of hydrolysis was found to be as good as a 24 hour hydrolysis period.

Eagle: Dr. Lyman, may we see your data about analysis for lysine according to the DNFB method?

Lyman: (Showed slide giving requested data.)

Eagle: Did you remove free gossypol before determining lysine content of CM-19?

Lyman: Yes, so that free gossypol could not interfere with the protein during hydrolysis.

Grau: Did you use the same method as Mrs. Conkerton for lysine determinations?

Lyman: Yes, but we hydrolyzed for 18 hours. We obtained the ε-DNP lysine after reaction with the DNFB for 24 hours instead of the 2 hours as in experiments of Mrs. Conkerton. Dr. Baliga will describe the procedure employed.

Baliga: The samples were reacted with DNFB for 24 hours, and then extracted with diethyl ether. The suspension was centrifuged and the residue was then hydrolyzed for 18 hours in 6 N Hydrochloric acid. The hydrolysate was subjected to vacuum distillation and then re-extracted with diethyl ether. Material was separated into a water-soluble fraction and a diethyl ether-soluble fraction. The water-soluble fraction was chromatographed on paper with a 1:1:1 mixture of butanol:water: acetic acid. After the paper was desolventized, the area containing the ε-DNP lysine was cut out. The ε-DNP lysine was eluted from the paper, and the ε-DNP lysine in solution was made to a specific volume. The amount of ε-DNP lysine was then spectrophotometrically determined. (Data presented at this junction by

Dr. Lyman for the lysine content of the cottonseed meals as determined by the microbiological assay method indicated no differences in their lysine levels.)

Frampton: Does the organism used in the microbiological assay of lysine have the ability to use the α-hydroxy analog of the β-keto analog of lysine?

Lyman: I do recall some work done along those lines years ago. However, I have no information on that subject.

Frampton: It is quite possible that analogs of lysine could arise in cottonseed meals due to conditions of processing and be used by the organism in the synthesis of lysine.

Holley: Do you know if gossypol reacts with the ϵ-amino groups of lysine in albumin? As I understand it, the ϵ-amino groups of lysine are sticking out from the albumin protein. Also, does the reaction take place better under moist conditions? One other point I should like to make is that about the conditions of raffinose in the cottonseed. How do we not know that raffinose is tied to the protein before the cottonseed is processed, and that the bond of binding is broken during processing? The effect of heat and processing conditions on the protein-raffinose reaction ought to be studied further.

Lyman: I agree with you. We think the ϵ-amino groups of lysine are ones affected by gossypol binding.

Couch: What is the accuracy of the microbiological assay method for determining the lysine content of cottonseed meals? Were your analyses obtained on samples of meal alone or on samples of the rations?

Lyman: Most lysine values can be repeated within 2% of themselves. The lysine contents of the meals were determined. Not very great differences were observed amongst meals subjected to the microbiological assay for lysine content.

Holley: Does albumin have more free amino groups than can be attributed to those of lysine alone?

Baliga: We found only lysine in the water-soluble phase. I would say it is responsible for free amino groups of albumin.

REACTION OF GOSSYPOL WITH CHEMICALS

W. H. King
Southern Regional Research Laboratory
New Orleans, Louisiana

Previous work conducted at this Laboratory on treatment of cottonseed with chemicals during processing has been directed toward developing information on procedures for reducing the gossypol content to nontoxic levels while maintaining the nutritive value of the protein. That work led to the development of the high-moisture, mild cooking procedure in the presence of acid or alkali, which, at optimum pH levels on both the acid, as well as the alkaline side, resulted in nontoxic meals of high protein solubility and high nutritive value, as measured by a large number of short-term chick feeding tests conducted by Dr. Watts. Tests made by Mr. Heywang on typical samples of the acid-cooked, neutral- and alkali-cooked meals showed that all caused egg discoloration when fed to laying hens.

Pilot plant work conducted in this Laboratory as well as in development laboratories of chemical machinery manufacturers and in cooperating commercial oil mills has shown that the procedure referred to can be carried out on a large scale in spite of certain mechanical difficulties arising from the plastic, dough-like state of the material encountered in one phase of the procedure. Although concerted efforts were made to increase the attrition so as to further reduce the active gossypol content of the resultant meals during the experimental work, none of the mechanical procedures tried reduced the "free" gossypol content to value less than 0.02%. The concensus of opinion among nutritionists who have been actively studying the problem is that reduction of "free" gossypol below 0.01% is necessary before the meals can be used (in unlimited quantity) in the rations of laying hens.

In view of the above results the study was returned to the Laboratory where numerous attempts were made to reduce the gossypol content by mechanical rupture of the glands followed by binding it to the meal by heat treatment or by extraction. Among the methods studied were procedures for ultimate comminution including passing the raw meats through an extremely high-pressure roller mill set at minimum clearance. This procedure completely deranged and smashed the pigment glands and denuded them of meal tissue. Homogenization of the wet meats in a Waring Blendor, extrusion through spring loaded pressure relief valves, set in tandem, at extremely high pressures, grinding in disc mills, beating in a hammer mill -- all were tried. In every instance, upon heat treatment under moist conditions which would have bound any liberated gossypol, the so-called "free" gossypol content of the finished meal was 0.02% or more.

Careful microscopic examination of the meals failed to reveal any evidence of unbroken pigment glands. The only way we were able to reduce the "free" gossypol content below 0.01% was by extraction with polar solvents. Yet when the raw meats were homogenized with polar solvents and refluxed for an extended period of time and the polar solvent removed by evaporation, without extraction, the finished meals still contained 0.02% or more of "free" gossypol.

These results led to a more careful examination of the "free" gossypol obtained on the aqueous acetone extraction of cottonseed meals, and to a determination of the gossypol in such extracts. The methods used have been published (J. Am. Oil Chemists' Soc. 34, 247 (1957)). Table I shows results of analyses of typical commercial cottonseed meals. It will be noted from the analytical results shown in the table that the gossypol content of commercial cottonseed meals may be as little as one-third of the material measured as "free" gossypol by the AOCS method.

Research work has continued on treatment of cottonseed with chemicals during processing. One promising result of this work was the elimination of gossypol below the 0.01% figure by treatment of the meats with stearyl amine during the high moisture treatment described previously. Addition of 2% of this substance to the reaction mixture in the presence of either acid or alkali, under conditions which have been found to result in highly nutritive cottonseed meals resulted in less than 0.01% of residual gossypol. Feeding tests conducted by Dr. Grau on this material show that it may be fed to laying hens in unlimited quantity without causing egg discoloration. Further work is being conducted along this line to determine the optimum conditions for use of fatty amines for this purpose.

TABLE I

RESULTS OF ANALYSES OF TYPICAL COMMERCIAL COTTONSEED MEALS

Type of Meal	"Free" Gossypol[a] (AOCS Method)	Gossypol	"Total" Gossypol[b] (AOCS Method)
	%		%
PPS	0.02	0.007	0.75
PPS	0.03	0.014	1.00
PPS	0.03	0.019	0.75
HSSS	0.03	0.013	1.00
Hydr.	0.10	0.055	1.10
Solv.	0.24	0.135	0.96
LSSS	0.02	0.007	0.62
LSSS	0.02	0.011	1.10
Aniline tr.	0.05	0.018	--

a/ Gossypol and "Gossypol-like" substances.
b/ Gossypol plus Gossypol derivatives.

Seminar IV - EFFECTS OF COTTONSEED PRODUCTS ON EGG QUALITY

Chairman - C. M. Lyman, Texas A&M College, College Station, Texas

STATUS OF RESEARCH ON EGG YOLK DISCOLORATION IN RELATION TO GOSSYPOL

C. R. Grau
Department of Poultry Husbandry
University of California
David, California

When gossypol is fed to laying hens, it is deposited in the yolk as gossypol-cephalin and as gossypol-protein. The amount of gossypol-cephalin is a sensitive measure of dietary gossypol that is available to the hen, and forms the basis for a definition of available gossypol units (AGU), as follows:

$$AGU = \frac{(Abs_{400} - Abs_{445})\ \text{diet egg} - (Abs_{400} - Abs_{445})\ \text{prediet egg}}{\text{percent of diet of material tested}} \times 100$$

Where Abs_{400} = Absorbance at 400 mμ of a crude cephalin solution as defined below,
Abs_{445} = Absorbance of solution at 445 mμ.

The crude cephalin solution is obtained by extracting 10 ml. yolk exhaustively with acetone, then extracting the residue once with 3:1 hexane-acetone and bringing the volume to 15 ml. This is read in a Beckman Model B spectrophotometer (for details see the Journal of Agricultural and Food Chemistry, 2, 982 (1954)). Each hen is its own control (prediet egg), and the bird is fed the diet for at least 10 days before a "diet egg" is studied.

Estimates of AGU are roughly correlated with "free" gossypol, but the biological method is much more sensitive. Eggs with absorbance differences of less than 0.025 (that is, diet egg absorbance minus prediet egg absorbance) have not been found to discolor on storage, or to darken in an atmosphere of ammonia when fresh. This statement applies only to cottonseed meals and to gossypol derivatives, not to apogossypol and its oxidation products.

When gossypol is heated with strong alkali, its carbonyl group is lost, and apogossypol is produced. This can no longer form a Schiff base with amines such as that with the aminoethanol of cephalin, but may still be incorporated into eggs, probably in protein combination. The phenolic groups of apogossypol, and its oxidation products, appear

to be responsible for the yolk discoloration, because blocking these phenolic groups by forming methyl ethers prevents discoloration in an ammonia atmosphere. Benzoate and oxyacetic acid derivatives also do not result in discolored yolks in ammonia, but these effects may be on molecular size rather than on blocking active groups. From a practical point of view, only alkali- and heat-treated soapstocks are involved in the apogossypol problem.

The most promising approach to the gossypol-in-egg problem is that of preventing its absorption from the intestinal tract, either by destruction or by binding into non-digestible linkages. Cooking and screw-pressing reduce the available gossypol drastically, but usually are not sufficiently effective to allow feeding significant amounts of meal to laying hens. Phloroglucinol can be used to destroy the gossypol, but this chemical is expensive and may reduce the nutritive value of the meal. Aniline does not bind gossypol tightly enough to prevent some hydrolysis and incorporation into the yolk. Some Schiff bases prepared from aliphatic amines by the Southern Utilization Research Branch are very promising as gossypol binders. One meal made from an aliphatic amine contained less than a tenth as much available gossypol as the best screw-pressed meals we have studied. This experimental meal could be fed safely to laying hens at any dietary level.

Discussion

Watts: Dr. Swensen of Louisiana State University published several years ago work concerned with the formation of a gossypol-iron-protein complex in eggs from hens fed gossypol. Have you studied the gossypol-iron or gossypol-iron-protein complex in the egg?

Grau: We have not been able to prove or disprove the work you mention. We find it very hard to get iron-deficient or iron-free eggs. We tried versene and other substances in efforts to reduce the iron contents of the eggs, but so far no method has been successful.

Holley: You stated that the aldehyde group of the gossypol molecule is the one involved in the transfer of gossypol through the chicken body and to the egg. How do you explain the ability of the compound, apogossypol, in which the aldehyde groups have been removed, to discolor eggs when fed to hens?

Grau: Apogossypol is apparently bound to protein, and is thus carried to the ovary and deposited in the yolk. The functional groups that are involved in the discoloration are primarily the phenolic groups; the aldehyde group is probably much less important.

Holley: Both the hydroxy and aldehyde groups of carotenoids are involved in their transfer to the chick egg. Do you think that gossypol passes through the chick body as free gossypol too, since there is some free gossypol in chicken eggs? If so, which substance, free or bound gossypol, discolors the egg yolk?

Grau: We have no evidence that free gossypol exists in the egg. We tried very hard to find some, but could not.

Heywang: At a level of 0.00025% free gossypol in the chick ration, I observed yolk discolorations. Yet, sometimes only a few of these turned green in an ammonia atmosphere.

Grau: It will be difficult to relate available gossypol to limits that can be permitted without risk of discoloration. Obviously, zero egg gossypol would be most desirable.

INCORPORATION OF GOSSYPOL INTO THE EGGS OF HENS FED GOSSYPOL SCHIFF BASES

J. M. Dechary
Southern Regional Research Laboratory
New Orleans, Louisiana

Eggs from hens fed gossypol Schiff bases derived from various aromatic and aliphatic amines were examined spectrophotometrically for gossypol-cephalin; the amount of this substance present in the egg yolk is a sensitive measure of the dietary gossypol that is available to the hen, and forms the basis for a definition of available gossypol units (AGU) as presented by C. R. Grau.

Aniline, p-aminobenzoic acid, dehydroabiethylamine, and cyclohexylamine were found to be ineffective gossypol binders, whereas the long chain aliphatic amines were very effective. Hens produced normal eggs when fed the 0.5% equivalent gossypol level in the diet of the Schiff base derived from n-octyl-, n-decyl-, n-dodecyl-, n-tetradecyl-, and n-octadecylamine.

Feeding studies were carried out by Dr. C. R. Grau of the University of California, Davis, California.

The chemical composition of the gossypol Schiff bases prepared are shown in Table I. Results from hen feeding studies are shown in Table II.

TABLE I

GOSSYPOL SCHIFF BASES

Schiff Base	% Yield	Recrystd. From	M.P. °C. (dec.)	Analyses[1] Carbon Calc'd	Carbon Found	Hydrogen Calc'd	Hydrogen Found	Nitrogen Cal'd	Nitrogen Found
Bis(furfurylimino) gossypol	100	---------	264-265	70.99	70.94	5.96	5.98	4.14	4.19
Bis(cyclohexylimino) gossypol	100	---------	314-315	74.09	73.55	7.70	7.76	4.12	4.05
Bis(oleylimino) gossypol	97	Isopropanol	106-109	77.91	77.63	9.91	9.78	2.75	2.75
Bis(n-octylimino) gossypol	93	---------	196-198	74.56	74.72	8.71	8.57	3.78	3.80
Bis(dehydroabietyl-imino) gossypol	95	Abs.Ethanol	212-214	79.81	79.34	8.42	8.60	2.66	2.62

1/ Analyses calculated on basis of two moles of amine condensing with one mole of gossypol and with the elimination of two moles of water.

TABLE II

AVAILABLE GOSSYPOL UNITS IN EGGS FROM HENS FED GOSSYPOL SCHIFF BASES

Gossypol Schiff Base	Millimoles Equivalent Gossypol	Percent of the Diet as Gossypol	AGU
Bis(phenylimino)gossypol	38.6	0.5	10.3
(Dianilinogossypol)	12.9	0.17	4.0
Bis(p-carboxyphenylimino)	11.2	0.15	35.5
Bis(benzylimino)	12.4	0.16	--2/
Bis(furfurylimino)1/	12.4	0.16	--2/
Bis(cyclohexylimino)1/	38.6	0.50	4.2
Bis(n-octylimino)1/	38.6	0.50	0.0
Bis(n-decylimino)	38.6	0.50	0.0
Bis(n-dodecylimino)	38.6	0.50	0.0
Bis(n-tetradecylimino)	38.6	0.50	0.0
Bis(n-octadecylimino)	38.6	0.50	0.2
Bis(dehydroabietylimino)1/	38.6	0.50	26.2-34.7
Bis(oleylimino)1/	6.2	0.08	--2/

1/ New compounds not previously reported and described in Table II.
2/ Dietary gossypol level was too low to permit a reliable measure of the gossypol-cephalin content of the egg.

EFFECT OF COTTONSEED OIL ON THE DISCOLORATION OF STORED SHELL EGGS

R. J. Evans
Michigan State University
East Lansing, Michigan

Previous work indicated that carotene, vitamin A, or ferric chloride might reduce the incidence of pink whites and salmon yolks in stored eggs produced by hens receiving a diet containing crude cottonseed oil. Carotene at levels of 2 or 4 grams per 100 pounds of mash, vitamin A acetate at levels of 4 or 8 million units per 100 pounds of mash, or 46 grams of ferric chloride per 100 pounds of mash did not reduce the incidence of pink whites or salmon yolks. The levels fed were similar to or higher than those that appeared to give some protection in limited preliminary trials.

Attempts have been made to fractionate the crude cottonseed oil and then separate out the material causing the pink whites and salmon yolks. Because crude cottonseed oil causes the discoloration and refined cottonseed (Wesson) oil does not, it was assumed that the causative agent might be removed with the soapstock. Removal of the soapstock did not reduce the incidence of discolored eggs, although the soapstock that was removed produced some egg discoloration. Neither commercial soapstock nor the acidulated soapstock produced any pink white discoloration, but there was evidence of slight gossypol discoloration of the yolks and some slightly salmon yolks. Ether extraction of acidulated soapstock appeared to concentrate the olive yolk factor in the extract.

Crude cottonseed oil was extracted with ethyl alcohol. Extracted cottonseed oil was as active in producing discolored eggs as was the crude oil. The alcohol extract produced no pink whites but gave a high incidence of early stages of olive yolks. An attempt was made to fractionate crude cottonseed oil by distillation under vacuum. The oil was heated until it smoked but very little distillate was obtained. The remaining oil was filtered to remove a precipitate that formed. None of the three fractions produced any discoloration. Either the factor causing discoloration was decomposed by heat or vaporized and was not collected. In Tables I and II are shown results of experiments conducted in 1955 and 1956.

TABLE I

PRODUCTION OF EGG DISCOLORATION BY FEEDING CRUDE COTTONSEED OIL OR ITS FRACTIONS, AND THE EFFECT OF CAROTENE AND VITAMIN A ON EGG DISCOLORATION - 1955

	% Pink whites	% Salmon yolks	% Olive yolks
Group 1			
Normal ration	0	0	0
Cottonseed oil	90	86	0
CSO + carotene	89	83	0
CSO - soapstock	96	89	0
Group 2			
Normal ration	0	0	0
Cottonseed oil	96	89	0
CSO + vitamin A	91	100	0
CSO soapstock	17	33	58

TABLE II

EFFECT OF CAROTENE, VITAMIN A, AND FERRIC CHLORIDE ON EGG DISCOLORATION FROM FEEDING COTTONSEED OIL, AND THE PRODUCTION OF DISCOLORATION BY DIFFERENT FRACTIONS OF CRUDE COTTONSEED OIL - 1956

	% Pink whites	% Salmon yolks	% Olive yolks
Group 1			
Normal ration	0	0	0
Cottonseed oil[1]	93	78	22
CSO + carotene	97	95	0
CSO + vitamin A	97	100	0
CSO + $FeCl_3$	90	40	33
Filtrate of CSO	0	0	0
Group 2			
Normal ration	0	0	0
Cottonseed oil[1]	83	63	37
CSO soapstock[2]	0	0	18
Ether insol. soapstock	3	9	24
Alcohol insol. CSO	73	50	50
Residue of CSO	0	0	0
Group 3			
Normal ration	0	0	0
Cottonseed oil[1]	67	33	44
Acid. soapstock[3]	0	28	19
Ether sol. soapstock	0	0	33
Alcohol sol. CSO	0	0	36
Distillate from CSO	0	0	0

1/ Crude cottonseed oil contained 0.06% gossypol. Refining loss was 5.3%.

2/ CSO soapstock contained 0.63% gossypol, 39.4% fatty acids, and 40.2% water.

3/ Acidulated soapstock contained 0.50% gossypol, 89.8% fatty acids, and 4.3% unsaponifiable matter.

FACTORS IN COTTONSEED MEAL THAT CAUSE PINK DISCOLORATION IN COLD STORAGE EGGS

A. R. Kemmerer and J. Masson
University of Arizona
Tucson, Arizona

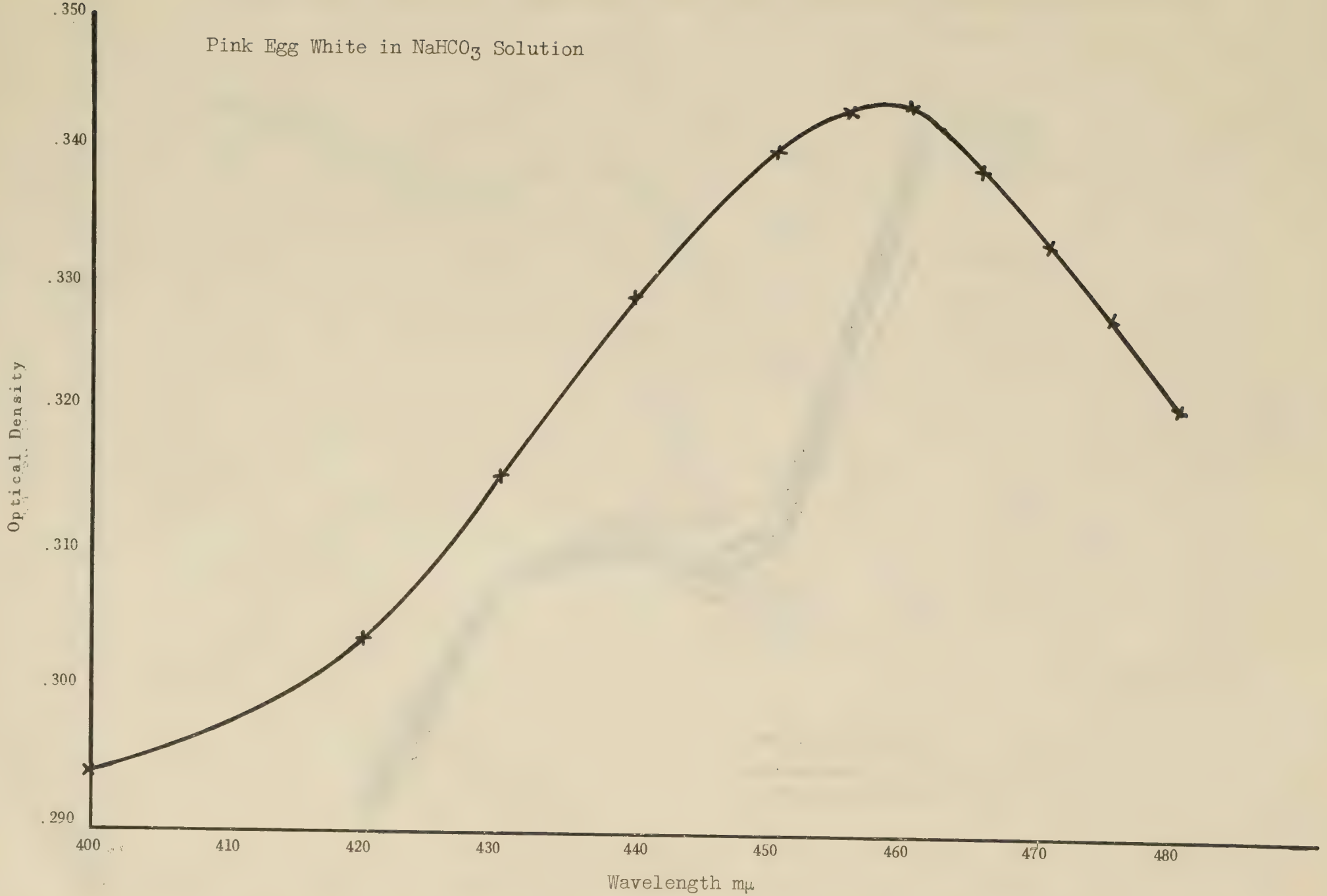
Fractions of crude cottonseed oil were fed to laying hens. The eggs were stored at approximately 45°F. for three to four months and then observed for pink discoloration. Results were as follows: (1) When the crude cottonseed oil was fed as 3% or 6% of the diet, pink discoloration resulted; (2) When the crude cottonseed oil was saponified and extracted with hexane or ethyl ether, neither of the extracts caused pink discoloration when they were fed at the equivalent of 6% crude cottonseed oil in the diets; (3) When the saponified material from (2) was acidified and re-extracted with hexane the hexane extract contained the pink discoloration factors; (4) When crude cottonseed oil was purified by heating with dilute sodium hydroxide, it still contained the pink discoloration factors.

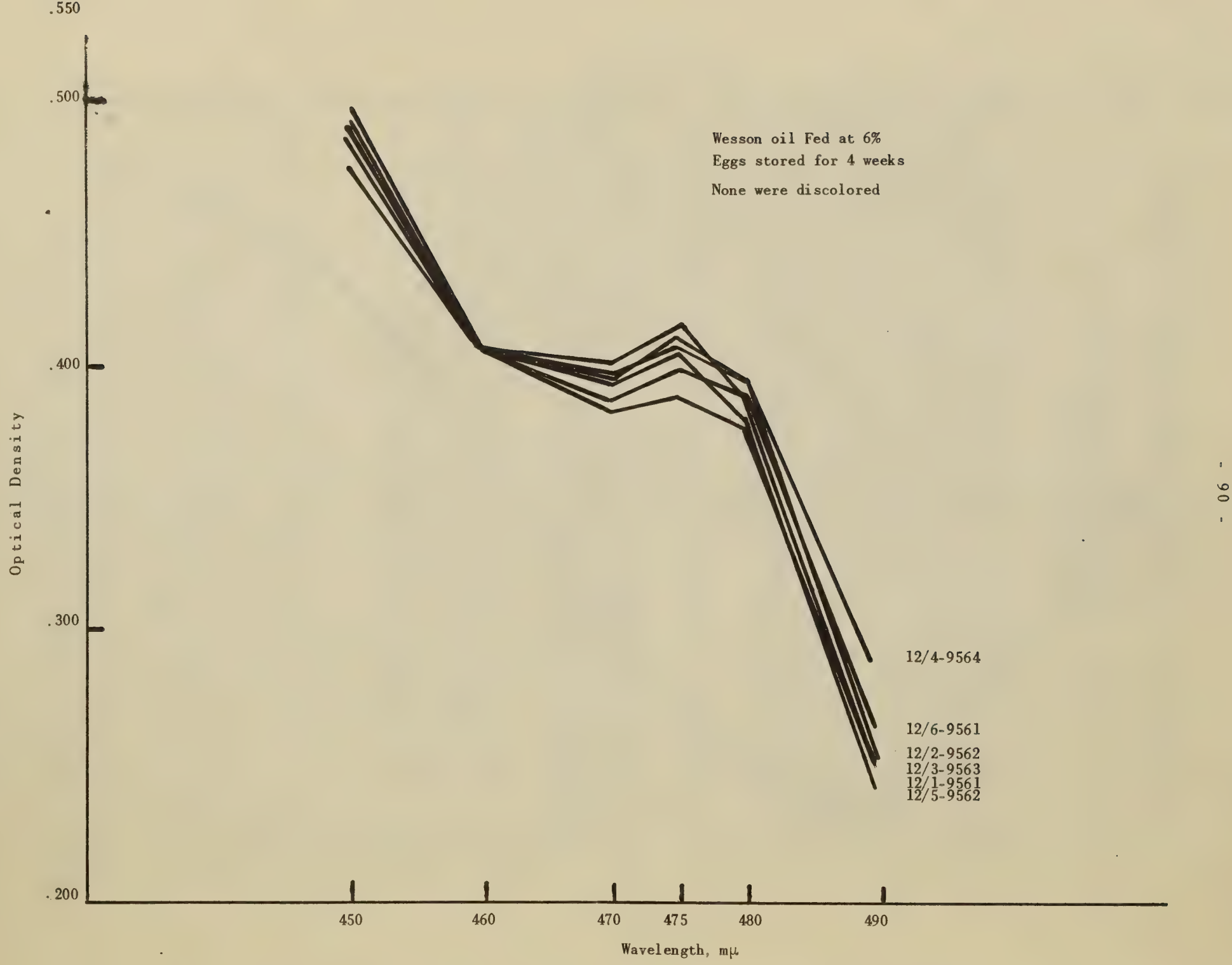
Apparently the pink discoloration factors are in the fatty acid factions of crude cottonseed oil.

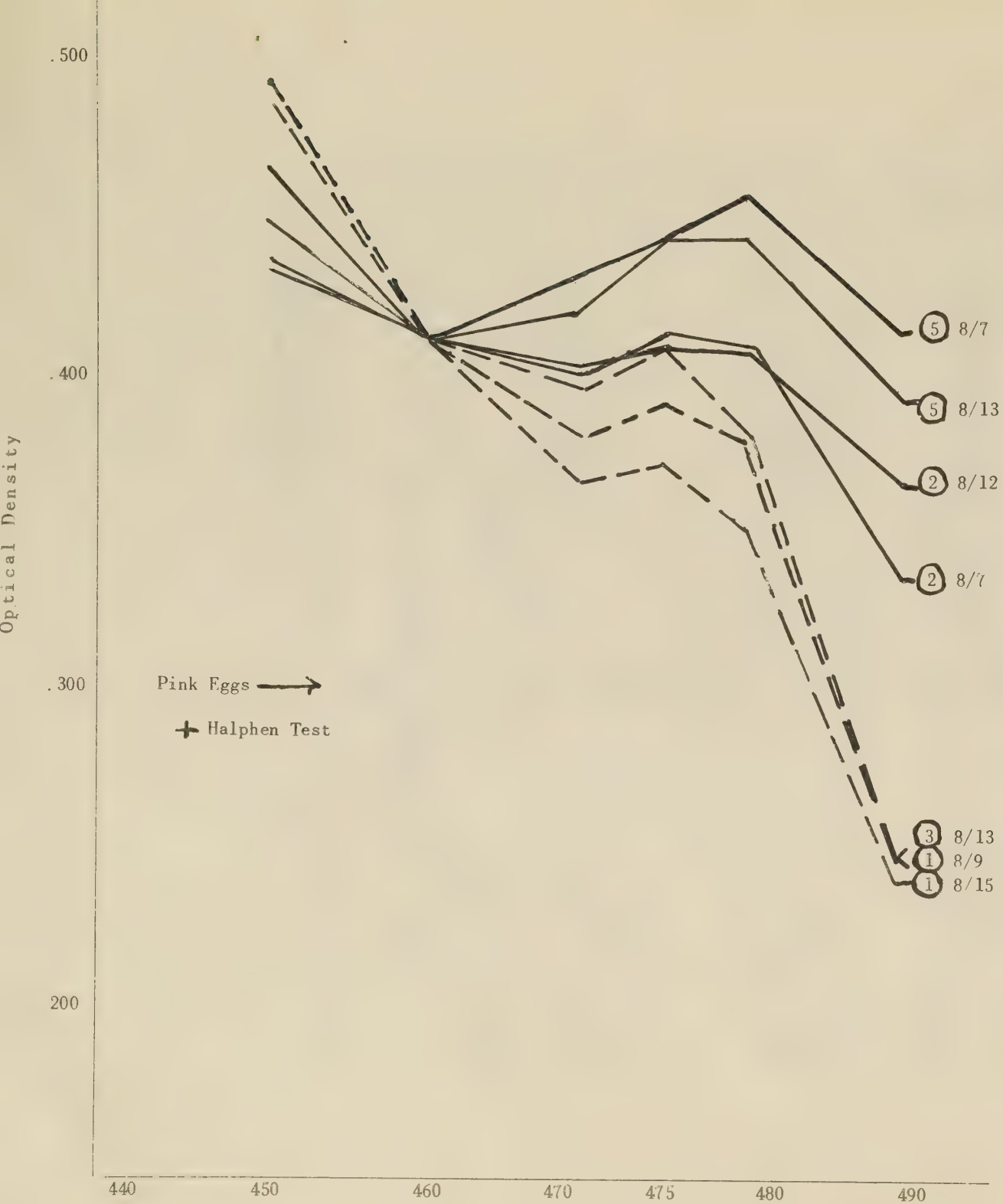
An attempt was made to correlate the Halphen test with the presence of the pink discoloration factors. Crude cottonseed oil gave a positive test. Pink discolored eggs gave a positive test but usually an orange color was obtained rather than the brilliant red that was obtained with the crude cottonseed oil. Eggs from hens fed Wesson oil gave varied results.

A spectrophotometric method was developed by which relative amounts of pink discoloration in eggs could be estimated.

Synthesis of sterculic acid is underway. It will be fed to laying hens and tested for pink discoloration properties.


Pink Egg White in $NaHCO_3$ Solution
.350
.340
.330
.320
.310
.300
.290
Optical Density
400
410
420
430
440
450
460
470
480
Wavelength mμ


.550
.500
.400
.300
.200
Optical Density
Wesson oil Fed at 6%
Eggs stored for 4 weeks
None were discolored
12/4-9564
12/6-9561
12/2-9562
12/3-9563
12/1-9561
12/5-9562
450
460
470
475
480
490
Wavelength, mμ



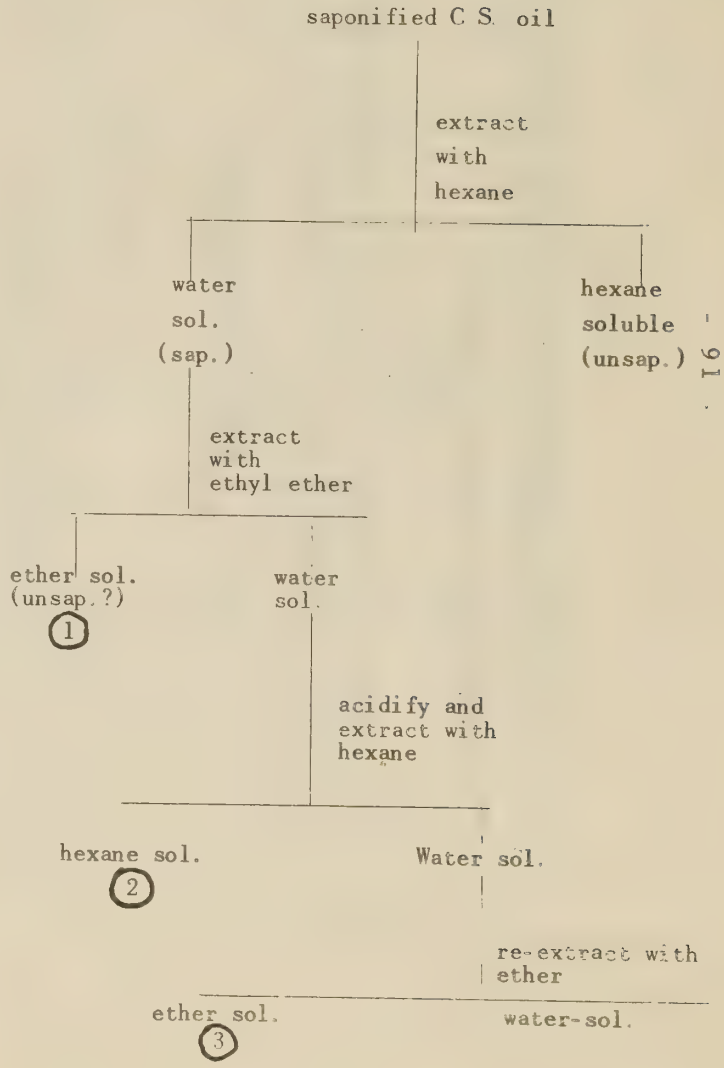
Halphen Reaction on discolored and normal egg yolk fat

(discolored are solid)

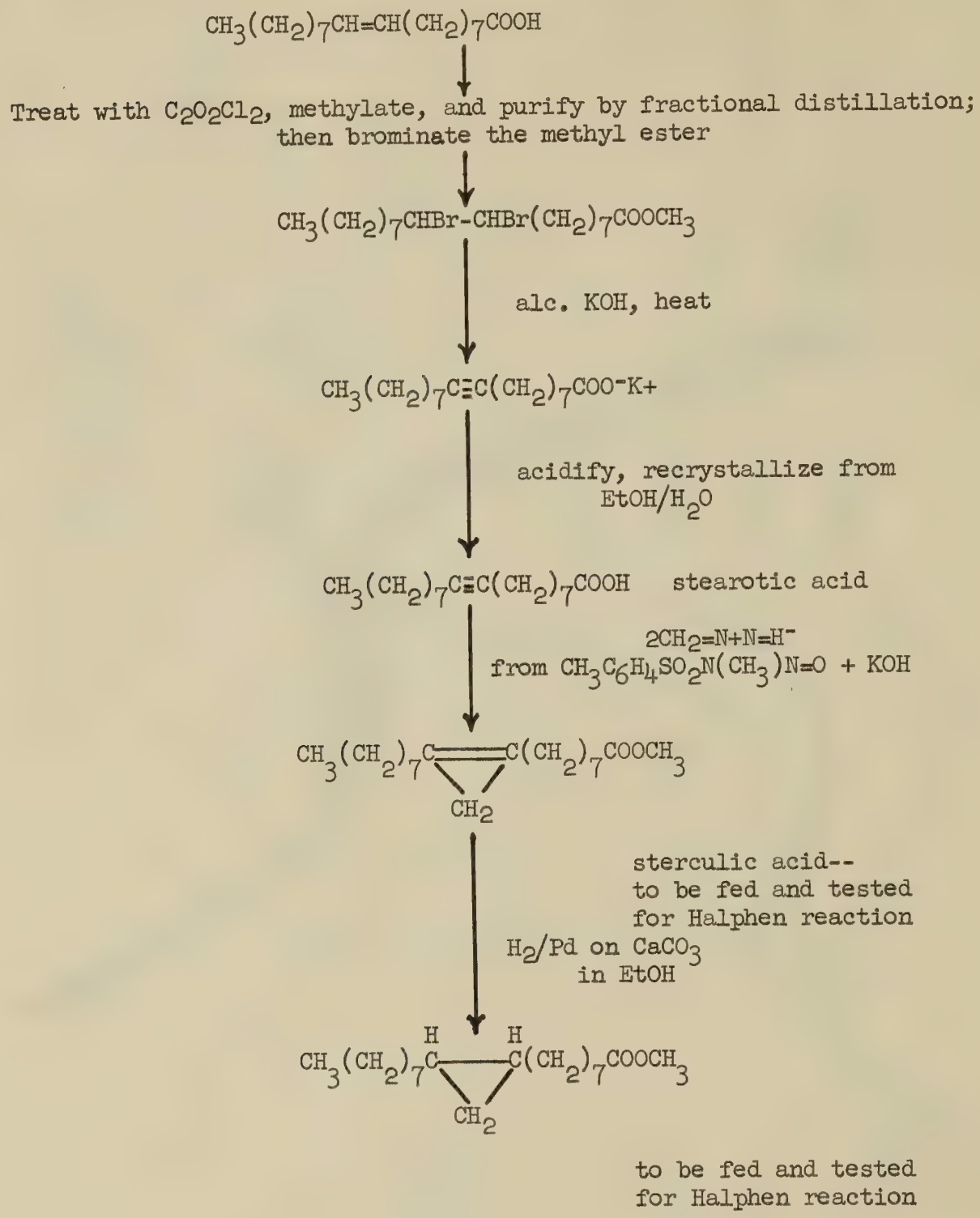
Storage 4 + months

Fraction from which eggs were obtained

(5) was crude C.S. oil



(PROPOSED) PREPARATION AND HYDROGENATION OF STERCULIC ACID



Seminar V - HISTOPATHOLOGICAL EFFECTS OF GOSSYPOL

Chairman - A. B. Watts, Louisiana State University, Baton Rouge, La.

HISTOPATHOLOGICAL EFFECTS OF GOSSYPOL

T. M. Ferguson, R. Narain, E. M. Banta and J. R. Couch
Departments of Poultry Science and Biochemistry and Nutrition
Texas A&M College
College Station, Texas

White Rock chicks, one day of age, were fed a synthetic diet containing various levels of gossypol, in the form of isolated cottonseed pigment glands, providing from 0.1 to 0.4% free gossypol. Gross and microscopic observations were made on these birds. Growth was depressed (187 gm. at 0.4% level at 8 weeks) and a lowered red cell count was found. The birds exhibited leg weakness and a general weakened condition. Other gross observations included an enlarged gall bladder, fluid in the pericardial and body cavities, a pale enlarged flabby heart and general body edema.

White Leghorn chicks, six to eight weeks old, reared on a practical diet were used to study acute toxicity of cottonseed pigment glands fed by capsule. Death occurred in from 24 to 64 hours when chicks were fed capsules providing 1.0 to 4.0 mg. free gossypol per gram of body weight. A hemoconcentration was observed in these chicks after administering the pigment glands by capsule.

Laying hens were fed capsules containing pigment glands, with varying amounts of free gossypol present, at periodic intervals during a 48 hour period. Fifty percent of the hens fed capsules supplying from 0.5 to 1.5 mg. free gossypol per gram of body weight were dead in 48 hours. Hens fed these levels of gossypol (pigment gland source) showed a breakdown of ova in the ovary and rupture of egg yolks, frequently accompanied by hemorrhage of the follicle. This was evident as early as 30 hours after capsules were fed. Accumulation of eggs in the reproductive tract and improper egg formation were observed. Hemoconcentration was evident in these birds as had been found in chicks given high levels of gossypol (pigment glands by capsule).

Preliminary gross and microscopic observations on these birds and on other hens fed pigment glands for various periods of time will be discussed.

HISTOPATHOLOGICAL EFFECTS OF GOSSYPOL FED TO PIGS

E. L. Stephenson
University of Arkansas
Fayetteville, Arkansas

Baby pigs were fed free gossypol in capsule at levels of 150 and 300 mg. per day. In both cases the material was fatal with both levels apparently being equally toxic. Post mortem examinations were made and the organs examined microscopically. Tissue samples were also taken for histological examination.

When the organs were examined the only one showing evidence of abnormalities was the kidney. These were covered with small peticulate hemorrhages and when opened were shown to contain larger hemorrhages. Microscopic examination revealed loss of staining properties in the areas of the tubules. This was interpreted to indicate tissue degeneration in these areas.

Discussion

Altschul: Stephenson, would you like to speculate on relationship between solubility and growth?

Stephenson: From my data there seems to be a definite relationship between nitrogen solubility and growth, and also between free gossypol and growth.

Curtin: What level of zinc was used to correct skin condition?

Stephenson: 40 mg., but this was not done in my experiment, but by someone else.

Earle: Have you performed any heart section studies?

Stephenson: We have sectioned kidneys and have heart tissue prepared for sectioning. On gross examination, however, we have observed no differences in the hearts of pigs fed diets containing cottonseed meal and those of the control group.

Earle: In our study with weanling pigs fed gossypol, every pig examined had an enlarged heart; some exhibited enlarged livers also in the acute and chronic stages. There was also evidence of edema in lungs and hearts.

Stephenson: Were you feeding gossypol as pure gossypol or gossypol in the form of cottonseed meal?

Earle: This was a study of cottonseed meal where the free gossypol content in the mixed diet was calculated from the gossypol content of the meal at the level we used.

Stephenson: We used pure gossypol and younger pigs, so maybe that may account for the differences. In our work we observed microscopic lesions in only the kidneys when pure gossypol was fed; however, when cottonseed meal was used, lesions were observed in many organs.

Seminar VI - RUMINANT STUDIES WITH COTTONSEED MEAL

Chairman - A. B. Watts, Louisiana State University, Baton Rouge, La.

THE EFFECT OF PROTEIN INTAKE ON REPRODUCTIVE PERFORMANCE, GAINS AND BLOOD CONSTITUENTS IN BEEF CATTLE

A. C. Warnick, E. Bedrak, M. Koger,
J. F. Hentges, Jr., and T. J. Cunha
University of Florida
Gainesville, Florida

Parous nonpregnant range cows supplemented with 1-1/2 pounds daily of cottonseed meal on reserve grass pasture had a 25 percent higher calf crop and calved 67 days earlier than comparable cows that received no supplement. One year following feeding there was an advantage on reproduction due to the cottonseed meal supplement. Two year old cross-bred heifers fed 1-1/2 pounds daily of cottonseed meal on grass pasture during the winter had an 18 percent higher calf crop and calved 32 days earlier than comparable heifers that received no supplement.

Twenty yearling British heifers were divided into four groups and fed individually various levels of protein for 160 days. Group 1 received 100% of N.R.C. level; Group 2, 64%; Group 3, 30%, and Group 4, 10% of the control ration. Equivalent amounts of energy, minerals and vitamins were offered each group. The average daily gain during the entire experiment for the respective groups was: 1, 0.9 pounds, 2, 0.7 pounds; 3, 0.0 pounds; and 4, 0.7 pound loss. The two lower levels of protein depressed appetite. The average percent hemoglobin at slaughter for the respective groups was 1, 11.4; 2, 11.8; 3, 9.4; and 4, 7.2. The average hematocrit at slaughter for the respective groups was: 1, 44.6 percent; 2, 43.5 percent; 3, 37.0 percent; and 4, 28.4 percent. The average percent total serum protein at slaughter for the respective groups was: 1, 6.7; 2, 6.8; 3, 6.3; and 4, 5.4. There were no detectable amounts of Xanthine Oxidase enzyme in the uteri of the heifers. The average Xanthine Oxidase enzyme activity based on microliters of oxygen uptake per milligram of nitrogen in liver tissue varied from 3.16 to 7.62 with much variability within groups.

None of the heifers in group 4 showed estrus or ovulated during the 160 days on experiment. There was some follicular activity on the ovaries. Only 2 of the 5 heifers in group 3 showed regular estrus periods while 3 heifers in group 2 and all 5 heifers in group 1 showed estrus before breeding began. Heifers were bred and slaughtered 44 days after breeding to determine embryonic development. All 5 heifers in group 1 settled on first service and had normal embryos. Two heifers in group 2 required a second service, but all had normal embryos. Neither of the 2 heifers bred in group 3 had embryos, and none of the heifers in group 4 showed estrus.

Discussion

Heidebrecht: Dr. Warnick, in your first experiment, were the diets of the cattle supplemented with vitamin A?

Warnick: No. The cattle were on pasture and under pasture conditions, this type of green grass, Panola, has no vitamin A deficiency. There is sufficient green grass to make supplementation unnecessary.

Heidebrecht: How do you account for the low percentage of pregnancy in cows in pasture in 1956 as compared to the 1955 test?

Warnick: This is an established characteristic obtained with Brahmin cows on pasture grass. There is an inhibition of lactation in these cows and they show alternate years of reproduction. This holds true over 3 and 4 years.

Heidebrecht: On clover, the cows have another and different degree of pregnancy - 100%; and 40% for others that were on grass. There's approximately 50-60% difference between grass and clover pasture cows. That's why I asked whether there was vitamin A supplementation. Could it possible be attributed to energy differences?

Warnick: What you say is true and it may be attributed, in part, to energy. We tried in our experiment to keep a minimum of variables.

Rusoff: What was the protein level of the grass and clover?

Warnick: The protein content of the clover was 20%; the protein content of the grass was 2%.

UTILIZATION OF PROTEIN AND NON-PROTEIN NITROGEN BY LAMBS

Walter R. Woods and A. D. Tillman
Animal Husbandry Department
Oklahoma Agricultural Experiment Station
Stillwater, Oklahoma

Protein Nitrogen

In the process of studying protein supplements for lambs it was found that cottonseed meal supported slightly lower daily gains and the lambs required more protein per pound of gain on cottonseed meals than on sesame meal or soybean-sesame meal combination. The rations contained, in percent, cottonseed hulls, 49.9; blackstrap molasses, 19.9; corn oil, 0.2; NaCl, 0.5; and added vitamin A-D. Calcium and phosphorus intake were equalized on the different rations. Either low nitrogen soluble cottonseed meal, high nitrogen soluble cottonseed meal, sesame meal or soybean meal-sesame meal were added in sufficient quantity to furnish protein for 8 percent protein ration with cerelose being added to bring the total to 100 percent. There was little difference between cottonseed meals of low nitrogen solubility (39.6%) and high nitrogen solubility (70.1%). The average daily gains and pound of protein required to produce a pound of gain for low nitrogen soluble cottonseed meal, high nitrogen-soluble cottonseed meal, sesame meal and soybean-sesame combination respectively were 0.26, 1.08; 0.30, 0.97; 0.34, 0.81; and 0.36, 0.82. In nitrogen balance trials following the growth trial, sesame meal fed lambs retained significantly more nitrogen than the lambs on the other supplements. The crude protein digestibility for the lambs on the soybean-sesame meal combination or sesame meal was significantly higher than for the cottonseed meals.

In trying to elucidate the reasons for the above results the following series of trials were initiated. Three levels of protein were supplied (4,6 and 8% protein rations) by each of cottonseed meal (hydraulic press), soybean meal (solvent extracted), and sesame meal. Two metabolism trials were conducted at each level of protein with 24 lambs. A total from 5 to 8 observations at each level of protein with each protein supplement was obtained. The rations were composed of, in grams, cellulose, 180; cottonseed hulls, 60; corn oil, 30; mineral mix, 30; vitamin A-D, 1; vitamin E, .012; and soybean meal, sesame meal or cottonseed meal in sufficient quantities to furnish enough protein for 4, 6 or 8% protein rations, with starch and cerelose being added in equal amounts to bring the total to 600 grams of daily feed intake.

The digestibility of cottonseed meal protein was significantly lower at all levels studied than either soybean or sesame meal protein. This was reflected in a slightly lower nitrogen retention. The crude protein digestibilities and nitrogen retentions for 4, 6 and 8% protein rations respectively for the various protein sources were: soybean meal 27.4, -0.77; 38.9, -0.19; and 58.4, 1.28; sesame meal 29.8, -0.64; 40.0, -0.01; and 52.4, 1.64; cottonseed meal 21.3, -0.88; 32.2, -0.30; and 43.7, 1.19.

In order to study the effect of the added cottonseed hulls in cottonseed meal the following metabolism trial was conducted. The basal ration was composed of, in grams, prairie hay, 500; cottonseed meal (hull free containing 57% protein), 88; $CaHPO_4$, 14; NaCl, 5; and vitamin A-D, 1. In ration 2, cottonseed meal was reduced to 41% protein by adding 38 grams of hulls. In ration 3 an iso-nitrogenous amount of soybean meal (108 gm.) was used in place of the cottonseed meal and in ration 4 the 38 gm. of cottonseed hulls were added to the soybean meal. A total of 6 lambs per treatment was used.

The addition of cottonseed hulls significantly reduced crude protein digestibility by about 3% when added to the cottonseed meal or soybean meal. Nitrogen retention reflected this decrease in digestibility. The addition of hulls also reduced the digestibility of crude fiber. The crude protein digestibility, crude fiber digestibility and nitrogen retention (gm) respectively for the different rations were: ration 1, 64.4, 67.4 and 2.03; ration 2, 61.4, 65.3 and 1.74; ration 3, 69.2, 68.2 and 2.25; ration 4, 66.2, 66.6 and 1.96.

Trials are in process now studying high quality cottonseed meal (chick tested) and low quality cottonseed meal as compared to a soybean meal basal ration.

Non-Protein Nitrogen

The efficient utilization of urea may require factors that are normally present in natural protein supplements. When iso-nitrogenous "purified rations" of soybean meal, dracket and urea (crystalline) were fed lambs the average daily gain was 0.26, 0.13 and -0.10 respectively. Two feeding trials involving a total of 60 individually fed lambs weighing about 50 lbs. were conducted to test the effect of "B" complex vitamins and soybean meal ash upon gains when consuming a purified diet. The basal diet contained the following percentage composition: cellulose, 40; starch, 25.6; cerelose, 25.6; minerals, 5.1; crystalline urea, 3.5; vitamin A-D, 0.1; choline chloride, 0.1, and vitamin E. In trial 1 the treatments were: (1) basal, (2) basal + soybean meal ash, (3) basal + "B" Complex vitamins, (4) basal + "B" Complex vitamins + soybean meal ash, (5) basal + corn oil,

(6) basal + corn oil + soybean meal ash. The daily gains (lb.) and feed consumption (gm) were: 0.02, 625; 0.18, 992; 0.14, 947; 0.21, 1053; 0.05, 607; 0.15, 828, respectively. In trial 2 the first four rations were repeated; ration 5 contained the basal + complete mineral mixture (equal to amount of soy ash). Ration 6 contained the basal + trace minerals (Mo, Br, and B). Daily gains and feed consumption values were: -0.03, 660; 0.06, 910; 0.05, 724; 0.08, 850; -0.04, 576; -0.08, 509; respectively. Soybean meal ash significantly increased gains when the trials were combined. Neither the additional complete mineral mixture nor the additional trace minerals increased gains. Preliminary trials comparing cottonseed meal ash with soybean ash as containing the growth promoting factors are being conducted. Further work on trying to determine the ash factor is underway at the present time.

Discussion

Curtin: Would the decreased protein solubility be due to poor digestibility of the added cottonseed meal hulls and to the poor protein quality of cottonseed hulls or crude fiber of the hulls?

Woods: That is a good possibility. That would explain, in part, lowered digestibility to crude fiber of the hulls of cottonseed meal, which are more digestible than that of hay.

Tillman: When we assumed there was no digestion of cottonseed meal hulls and calculated on the basis of no digestibility of cottonseed meal hulls, we still had reduction.

COMMITTEE REPORTS

REPORT ON COOPERATIVE COTTONSEED MEALS EXPERIMENT

The purpose of this experiment was stated as follows:

"To provide information regarding the suitability and performance of mixtures of commercial cottonseed meal with soybean meal in practical poultry and swine rations, and to provide additional information on the relationship between processing conditions and protein quality."

The following conclusions may be drawn:

1. All of the meals and all of the rations have been analyzed chemically; there is a variation in their chemical composition. These analyses show that the free gossypol content of meals is affected by the process used in manufacturing the meal. The free gossypol content of meals prepared by the screw-press process, the pre-press solvent extraction process, and solvent extraction followed by chemical treatment is 0.04% or below. Meals with free gossypol content higher than 0.04% were produced by the hydraulic press and straight solvent extraction processes. Aside from one meal in the group, all of the meals that had been analyzed had a nitrogen solubility less than 75%. Chemical properties of the meal such as nitrogen content, nitrogen solubility, total gossypol, phosphorus, total fat , total sugar, reducing sugar, lysine content and crude fiber content, cannot be predicted on the basis of the process used but are a function of processing conditions.

2. It is recommended that the nutritional data obtained in this experiment be subjected to complete statistical analyses with consultation, wherever necessary, with competent bio-metricians before any conclusions be drawn about the relationship between processes, processing conditions, chemical properties and nutritive performance of the meals for broiler growth.

When this statistical analysis is completed, information should be available on the suitability of present commercial production of cottonseed meal in practical broiler rations and in mixtures with soybean meal.

It is possible that information will also be available on the effect of free gossypol content, fiber content, total gossypol content, nitrogen solubility, lysine content, and other chemical characters on the nutritive performances of the meals alone and in mixtures with soybean meals in practical broiler rations. There should also

be provided information on the comparative performance of these meals in various stations and after various periods of storage in handling.

3. This comprises probably the greatest single large scale cooperative nutrition experiment under controlled conditions. It should provide an excellent basis for estimating the present status of cottonseed meals in broiler rations and provide leads for future research to improve this status.

All those who participated in this experiment should be congratulated on their performance. The conference records its gratitude to Dr. V. L. Frampton for his assuming the major burden of responsibility for this program and for his competent efforts.

Report of Committee on Status of Cottonseed Meal in Poultry Rations

A. M. Altschul, Chairman
J. Corbin
C. A. Denton
J. M. Dechary
H. D. Fincher
C. R. Grau
R. Stokes
J. Waddell
A. B. Watts
W. S. Wilkinson

The Committee reaffirmed the validity of the statement on cottonseed meal* made at the Third Conference on Cottonseed Processing and Nutritive Value of the Meal held in New Orleans, November, 1953, insofar as it refers to poultry.

The Committee feels that there is evidence that there exist cottonseed meals of 0.04% free gossypol but with less than 75% nitrogen solubility, which when substituted for 50% of the soybean meal in a practical broiler ration, give excellent performance. The Committee feels, however, that it should be the objective of cottonseed processors, when preparing meals for broiler rations, to retain the maximum quantity of desirable nutrients insofar as possible. It is the general experience that with any given process, higher nitrogen solubility generally reflects less damage to or less destruction of desirable nutrients.

Recommended Research on Poultry:

The Committee recommends the following lines of research as needed to answer some of the questions that have been raised at this Conference:

1. Cottonseed meals representative of the best production should be made available to research investigators to study their performance in mixtures with soybean meal in broiler rations. The cottonseed meals to be tested should have the same protein content as the standard soybean meal used for comparison; the 50% meal will probably be most desirable.

* The statement reads as follows: "results presented thus far indicate that chick and broiler rations containing cottonseed meal and soybean meal in equal proportions on a nitrogen basis are equal or superior to rations based on either cottonseed meal or soybean alone, when the cottonseed meal used has 0.04% or less of free gossypol and 75% or more of nitrogen solubility in 0.02 N NaOH solution."

2. The various lysine analyses that have been made on cottonseed meal seem promising as a means for identifying meals of higher nutritive value. The chemical work on the lysine content of the meals and correlation between these data and nutritional performance should be intensified.

3. Fundamental investigations on the reactions of isolated cottonseed proteins with other constituents of the meal such as gossypol, carbohydrates and phosphorus compounds should be pursued. These offer the promise of providing explanations for the reduction of nutritive value that may take place during processing.

4. As a result of fundamental investigations on the chemistry and structure of gossypol, there is now a clearer understanding of the nature of the materials that are responsible for egg yolk discoloration. Moreover, there are now available several chemical approaches for removing this material from cottonseed meal. Investigations of these approaches should be pursued vigorously in the laboratory and in commercial mills wherever feasible and desirable, and the work on the reactions of gossypol, which is the basis for the progress that has been made, should be continued.

5. The egg white discoloration problem is an important problem particularly because certain cottonseed oil byproducts are used in some oilseed meals. Research on this problem and on the materials in cottonseed oil, particularly the "Halphen" acid should be continued.

Report of Committee on Swine and Ruminants

A. Allen Heidebrecht, Chairman
I. P. Earle
E. R. Barrick
L. V. Curtin
K. T. Holley

Swine

Factors that have been shown to affect the tolerance of growing swine for gossypol are protein quality, level of dietary protein, and breeding.

Available data indicate that a dietary level of 0.0045% free gossypol can be fed without evidence of toxicity. A level of .0066% is the lowest dietary level that has been reported to be toxic.

Adequate data are not available on the value of bound gossypol and nitrogen solubility as measurements of cottonseed meal quality for swine.

Recommendations:

Further work should be conducted on cottonseed meal to: (a) determine gossypol tolerance under well-defined conditions; (b) determine the effect of bound gossypol on feeding value; (c) study the effect of crude fiber on feeding value; (d) study the value of nitrogen solubility as a measure of feeding value; (e) study the mode of action of gossypol as related to toxicity; and (f) study the use of cottonseed meals in breeding type rations.

Ruminants

Further evidence was presented to show that cottonseed meal can be fed as the only source of supplementary protein for growth and reproduction of ruminants with satisfactory results.

Recent growth and metabolism studies indicate that differences exist among cottonseed meals and other vegetable protein supplements as sources of protein for lambs.

Recommendations:

Further work should be conducted to determine if differences exist among cottonseed meals as sources of protein for calves, beef cattle, and dairy cattle.

The observed effect of fiber in lowering the digestibility of proteins as well as other possible factors affecting differences in digestibility that have been demonstrated with lambs should be investigated further.

Report of Committee on Chemical Measurements and Nutritive Value

C. M. Lyman, Chairman
V. L. Frampton
H. E. Robinson
P. D. Aines
C. L. Hoffpauir
H. L. Wilcke

Investigations to date indicate that there are a number of chemical determinations which tend to measure differences in the nutritive value of cottonseed meals, but none of these gives an entirely satisfactory evaluation when used as the sole criterion. Chemical determinations discussed at this conference include:

1. Gossypol

Evidence was presented to show that increasing levels of total gossypol (0.62% to 1.11% as determined in the collaborative experiment) have a detrimental effect on the rate of growth of chicks.

(a) Bound gossypol* -- In those cottonseed meals in which free gossypol is low, the "bound" gossypol is the major damaging factor.

(b) Free gossypol -- In those cottonseed meals which have a high free gossypol content, the free gossypol is the major damaging factor.

2. Nitrogen Solubility

For intermediate levels of nitrogen solubility, there was no direct relationship between this criterion and nutritive value. No conclusive evidence was presented to justify a change in the previous recommendation that cottonseed meals having 0.04% or less of free gossypol and 75% or higher nitrogen solubility in 0.02 N sodium hydroxide solution are satisfactory for use in chick rations.

Recommendations for Future Work

1. It is recommended that work be continued and expanded for the development of improved chemical procedures for evaluating protein quality and heat damage in samples of cottonseed meal.

* Bound gossypol is defined as the difference between total gossypol and free gossypol as determined by the standard AOCS procedures, within the limits of the scope of these methods.

2. Total lysine as determined by the Moore and Stein method was found to be positively correlated with the nutritive value of the meals used in this collaborative test. Further studies should be conducted on the relationship between the lysine content and the nutritive value of cottonseed meals.

3. It is recommended that an investigation designed to develop a simplified procedure for the determination of lysine be conducted.

4. It is recommended that studies be conducted on the possibility of using the determination of free lysine epsilon amino groups as a measure of the nutritive value of cottonseed meals.

5. Additional research is recommended to characterize the different compounds measured by total and/or free gossypol determinations, and the effect of these compounds on the nutritive value of cottonseed meal.

6. An extension of investigations to determine the effect of processing on amino acids is recommended.

Report of Committee on Effect of this Research on Industry Development

G. A. Harper, Chairman
H. J. Konen
P. A. Williams
G. E. Goheen
J. F. Moloney
C. W. Upp

This program is making significant progress toward enabling cottonseed meal to compete effectively as a protein concentrate for poultry and swine. This protein concentrate market amounts to an estimated 3,000,000 tons in cotton states.

The information developed by this program has caused widespread re-evaluation of the nutritional properties of cottonseed meals by research workers, cottonseed processors, feed manufacturers, and feeders. It has resulted in their recognition of the important contribution which cottonseed meal can make to the poultry and swine industries.

Excellent cooperation and coordination among many research groups and the industry have been responsible for the splendid progress of the program. Research leaders recognize it as an outstanding example of united effort.

The conference renders effective service to cottonseed processors, feed manufacturers, and consumers by making available information developed through research.

Data presented at this conference indicate the need for further research on the desirability of high-protein cottonseed meal for poultry and careful study by cottonseed processors of practicality of producing high-protein meal.

This conference should stimulate cottonseed processors to increase their efforts to improve the quality and uniformity of commercial meals and to make more extensive use of any quality criteria which have been established by conclusive research data.

Studies reported here emphasized again that processing conditions which employ minimum heat produce meal of highest nutritive value, provided the free gossypol content is reduced below the tolerance level of the animals fed.

The conference developed information on several promising measures of nutritive quality which processors and feed manufacturers should investigate as methods of product evaluation.

APPENDIX

List of Attendance

Aines, P. D., Buckeye Cellulose Corporation, Cincinnati, Ohio
Baliga, B. P., Texas Agricultural Experiment Station, College Station, Texas
Barnett, B. D., The Clemson Agricultural College, Clemson, S. C.
Barrentine, B. F., Mississippi State College, State College, Miss.
Barrick, E. R., North Carolina State College, Raleigh, N. C.
Bredeson, D. K., French Oil Mill Machinery Co., Memphis, Tenn.
Brown, P. B., Agricultural Experiment Station, Louisiana State University, Baton Rouge, La.
Bucek, O. C., Dow Chemical Company, Lake Jackson, Tex.
Burns, W. A., Ranchers Cotton Oil Company, Fresno, Calif.
Cabell, C. A., Agricultural Research Service, Beltsville, Md.
Carroll, R. W., Quaker Oats Company, Barrington, Ill.
Cavanagh, G. C., Ranchers Cotton Oil Company, Fresno, Calif.
Coleman, W. T., Western Cottonoil Company, Abilene, Tex.
Combs, G. E., University of Florida, Gainesville, Fla.
Corbin, J. E., Ralston Purina Company, Checkerboard Square, St. Louis, Mo.
Couch, J. R., Texas A&M College, College Station, Tex.
Curtin, L. V., McMillen Feed Mills, Decatur, Ill.
Day, E. J., Mississippi State College, Agricultural Experiment Station, State College, Miss.
Denton, C. A., Poultry Nutrition, Agricultural Research Service, Beltsville, Md.
Deobald, H. J., Plains Cooperative Oil Mill, Lubbock, Tex.
Durham, W. A., Sr., Tristate Blow Pipe Company, New Orleans, La.
Eagle, E., Swift and Company, Union Stock Yards, Chicago, Ill.
Earle, I. P., Agricultural Research Service, Beltsville, Md.
Evans, R. J., Agricultural Experiment Station, Michigan State University, East Lansing, Michigan
Ferguson, T. M., Agricultural Experiment Station, Texas A&M College, College Station, Tex.
Fincher, H. D., Anderson, Clayton & Company, Houston, Texas
Gandy, D. E., National Cottonseed Products Association, Ruston, La.
George, J. P., Crenshaw Oil Company, Inc., Crenshaw, Miss.
Grau, C. R., Agricultural Experiment Station, University of California, Davis, Calif.
Harper, G., National Cottonseed Products Association, Dallas, Tex.
Heywang, B. W., Southwest Poultry Experiment Station, Agricultural Research Service, Glendale, Arizona
Heidebrecht, A., Western Cottonoil Company, Abilene, Tex.
Holley, K. T., Georgia Agricultural Experiment Station, Experiment, Georgia
Hollowell, E., National Cottonseed Products Association, Atlanta, Ga.

Houghtlin, R. G., National Soy Process Association, Chicago, Ill.
Hundley, J. M., United Nations, UNICEF, New York City, New York
Kemmerer, A. R., Agricultural Experiment Station, University of Arizona, Tucson, Arizona
Konen, H. J., Uncle Johnny Mills, Houston, Tex.
Lewis, K., National Cottonseed Products Association, Lubbock, Texas
Lucas, R. M., Chickasha Cotton Oil Company, Chickasha, Okla.
Lyman, C. M., Texas Agricultural Experiment Station, Texas A&M College, College Station, Texas
McKnight, F. G., Western Cottonoil Company, Abilene, Tex.
Martin, J. B., Procter and Gamble, Miami Valley Laboratories, Cincinnati, Ohio
May, R. W., The Union Oil Mill, Inc., West Monroe, La.
Moloney, J. F., National Cottonseed Products Association, Memphis, Tenn.
Moore, W. B., Cotton Gin and Oil Mill Press, Dallas, Tex.
Morgan, I., Jr., Morgan Oil and Refining Company, Farmville, N. C.
Roberts, B. J., Traders Oil Mill Company, Fort Worth, Tex.
Robinson, H. E., Swift and Company, Union Stock Yards, Chicago, Ill.
Roots, L. C., Anderson, Clayton and Company of Mexico, Uruguay, Mexico, D. F.
Rusoff, L. L., Dairy Dept., Louisiana State University, Baton Rouge, La.
Ryan, C. E., J. T. Gibbons, Inc., New Orleans, La.
Sanders, C., Producers Cotton Oil Company, Fresno, Calif.
Smith, F. H., North Carolina State College, Raleigh, N. C.
Stephenson, E. L., University of Arkansas, Agricultural Experiment Station, Fayetteville, Ark.
Stokes, R., Buckeye Cellulose Corporation, Cincinnati, Ohio
Tillman, A. D., Agriculture Experiment Station, University of Tennessee, Knoxville, Tenn.
Upp, C. W., Louisiana State University, Baton Rouge, La.
Waddell, J., E. I. duPont deNemours Company, Wilmington, Del.
Warnick, A. C., Agricultural Experiment Station, University of Florida, Gainesville, Fla.
Watts, A. B., Agricultural Experiment Station, Louisiana State University, Baton Rouge, La.
Wilcke, H. L., Ralston Purina Company, St. Louis, Mo.
Wilkinson, W. S., Louisiana State University, Baton Rouge, La.
Williams, P. A., Southern Cotton Oil Company, New Orleans, La.
Woods, W. R., Animal Husbandry Department, Oklahoma A&M College, Stillwater, Okla.
Staff Members of the Southern Regional Research Laboratory, Southern Utilization Research and Development Division, New Orleans, La.